Animal Reproduction and Artificial Insemination

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राष्ट्रीय शैक्षिक अनुसंधान और प्रशिक्षण परिषद्
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Foreword

The National Policy on Education (1986) while suggesting various thrust areas, lays importance on vocationalization as this is crucial in the proposed educational reorganization It is meant to enhance individual employability, to reduce the mis-match between the demand and supply of skilled manpower, and to provide an alternative for those pursuing higher education without any particular interest or purpose It also, helps in linking education with the productivity and economic development of the country

In view of the above, the NCERT is making a renewed all-out effort to provide necessary academic support to the implementing agencies in the States/Union Territories One of the major contributions of the NCERT in Vocationalization programmes is in the field of curriculum development and in the development of model instructional materials The materials are developed through workshops and training programmes in which experts, subject specialists, curriculum framers and teachers of the vocational course are involved

The present book on 'Animal Reproduction and Artificial Insemination' is an outcome of the teachers' training programme organized by the NCERT for +2 vocational teachers teaching Dairying and allied vocational courses, in which a group of experts delivered the lectures The same were compiled and brought out in a form of reference book in the first stage, which was later reviewed, updated and modified by a group of experts in the light of the NCERT syllabus in a workshop The book is being published for wider dissemination amongst students and teachers throughout the country I hope they will find the book useful

I am grateful to all those who have contributed to the development of this book I must also acknowledge the immense interest taken by Prof A K Mishra, Head, Department of Vocationalization of Education in inspiring his colleagues in their endeavour to develop instructional materials. Dr. A.K. Sacheti, Reader, functioned as the project coordinator for the development of this title. He has my appreciation and thanks for planning, designing, organizing the training programme, conducting the workshop, for technical editing and for seeing this book through the Press

Suggestions for improvement of this book will be welcome

P.L. MALHOTRA
Director
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New Delhi

Preface

Ever since the introduction of vocationalization in our school system by several States and Union Territories in our country, the paucity of appropriate instructional materials has been felt as one of the major constraints in the implementation of the programme and a source of great hardship to pupils offering vocational studies at the higher secondary stage

The Department of Vocationalization of Education of the National Council of Educational Research and Training, New Delhi has started a modest programme of developing instructional materials of diverse types to fill up this void, in all major areas of vocational education The task is too gigantic to be completed by any single agency but the model materials being developed by us might provide guidance and impetus to authors and agencies desiring to contribute in this area

This book is meant for the pupils offering Dairying and allied vocational courses It is also suitable for the teachers teaching such courses The book has four parts· (i) Reproductive Physiology (ii) Artificial Insemination (iii) Reproductive Management (iv) Lactation. Each part has several chapters In all there are 31 chapters All these make a comprehensive book on Animal Reproduction and Artificial Insemination It is hoped that the users will find this book immensely useful

The present book is the outcome of a teachers' training programme organized by the NCERT for +2 vocational teachers teaching Dairying and allied vocational courses in collaboration with Krishi Vigyan Kendra, NDRI, Karnal in which a group of experts delivered the lecture(s) The same were compiled and brought out in a form of reference book for distribution amongst the teachers The compiled version of the book was reviewed, updated and modified by a group of experts in the light of the NCERT syllabus, in a workshop conducted by the NCERT in collaboration with the Division of Dairy Extension, NDRI, Karnal. The names of the experts whose lecture notes were considered in the review workshop and who actually reviewed, updated and modified are mentioned elsewhere and their contributions are admirably acknowledged Our thanks are due to Dr. R L Kherde, Head, Krishi Vigyan Kendra and Trainers' Training Centre and later on Division of Dairy Extension, NDRI, Karnal for acting as Course Director in both the programmes and for providing all the necessary facilities in organizing the same Our thanks are also due to Shri C.A. Thomas, Scientist-2, Krishi Vigyan Kendra for assisting in organizing the short term teachers' training programme and for bringing out the reference book in cyclostyled form Dr A.K. Sacheti, Reader and Coordinator of this project, Department of Vocationalization of Education, deserves special thanks for planning, editing and bringing the book to its present form The assistance of all in the Krishi Vigyan Kendra and Division of Dairy Extension, NDRI, Karnal and the Department of Vocationalization of Education, NCERT, is also gratefully acknowledged.

DR ARUN K. MISHRA
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The following experts delivered the lecture(s) during the short-term teachers' training programme organized by the NCERT The handouts given by them were compiled in the form of reference book on Animal Reproduction and Artificial Insemination and the same was made available to the trainees Their contribution is gratefully acknowledged

Dr M L Madan, Dr G C Jain, Dr S V Pachalag, Dr B S Prakash, Mr. V S Raina, Prof. R S. Pande, Dr S K Sharma, Dr. R S. Ludri, Dr P K Nagpal and Prof M V N Rao

The following experts reviewed and made necessary additions and alterations in the compiled book on Animal Reproduction and Artificial Insemination in a workshop conducted by the NCERT Their participation as reviewers is gratefully acknowledged·

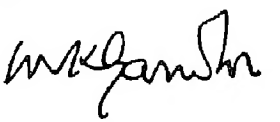
Dr. M L. Madan, Dr J D Honmode, Dr S B Kodagali, Dr G C. Jain, Dr S V Pachalag, Dr P V Sarma, Dr B S Prakash and Mr V S Raina

GANDHIJI'S TALISMAN

"I will give you a talisman. Whenever you are in doubt or when the self becomes too much with you, apply the following test :

Recall the face of the poorest and the weakest man whom you may have seen and ask yourself if the step you contemplate is going to be of any use to him. Will he gain anything by it ? Will it restore him to a control over his own life and destiny ? In other words, will it lead to Swaraj for the hungry and spiritually starving millions ?

Then you will find your doubts and your self melting away."



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PART I

REPRODUCTIVE PHYSIOLOGY

CHAPTER ONE

Concepts in Animal Reproduction

During the course of mammalian evolution, there have been notable anatomic, endocrinologic and physiological changes. Among the more obvious are: economy in production of gametes, reduction in the size of egg, internal fertilization, development of corpus luteum (CL) as an endocrine gland, development of placenta as a nutritive, excretory, endocrine and protective organ and finally, birth at a time when young ones can survive in the environment provided for them.

The animals which man has domesticated over the centuries to meet his needs of food, clothing, power and companionship include cattle, buffaloes, sheep, goats, pigs, horses, cats, dogs and poultry. These animals differ with respect to sexual season, sexual cycle, gestation and lactation period, placentation and litter size.

The reproductive cycle in each of these animals is regulated by interactions between the central nervous system, hypothalamus, hypophysis and gonads. Each of these body systemic components have to function individually and collectively to bring about functional reproductive rhythm. Man, during the process of domestication, has exploited each of these animal species in order to maximize returns in economic terms. Man, by himself, is a poor creature in many of the faculties and systems when compared to the animals he has domesticated, but he has oriented himself in such a way that his faculty of coordination has enabled him to attain a position whereby he could optimize animal productivity.

The biosphere of the earth has an excellent ecological chain system through which life is sustained. Energy from the sun passes through the living system, plants, animals and man and this regulates life. Ecological symbiosis of the plant, animal, man system, puts animals into a unique position, where, on the one hand it utilizes plants for its own growth, and on the other, generates milk, meat, wool and eggs for utilization by man. Increased multiplication of animal life through increase in number may seriously limit the earth's resources but increase in unit production of milk, meat, wool and eggs would ideally sustain the ever growing human demands. Under these circumstances, high productivity from each animal has become highly imperative. Rapid multiplication of these

high productive species, therefore, will involve bringing about innovative advances in their reproductive patterns. As there can hardly be production without reproduction, the need of the hour, therefore, is to optimize the reproductive mechanisms.

The efficiency of reproduction in a given species depends on the length of sexual season, frequency of estrus, number of ovulations, duration of pregnancy, litter size, suckling period, puberty age, and duration of the reproductive period in the animal's life. This efficiency may decline as a result of seasonal, genetic, nutritional anatomic, humoral or pathogenic factors. These factors may also result in partial or complete reproductive failure. The main objective of every animal husbandry worker should be to control and prevent such failures. Several new technologies have been developed to enhance fertility and productivity among animals, particularly, bovines.

Fertility in cows and bulls depends upon a combination of factors and ultimately on the efficiency of artificial insemination programmes. The different parameters of fertility are puberty, sexual desire, non-return rate, conception rate, calving rate, services per conception or index of pregnancy, calving interval, longevity or working life and perinatal calving problems.

Understanding of the process of reproduction and artificial insemination involves a thorough knowledge of the events of gamete production, organs involving gamete production and factors which control them; fertilization processes and gestation and parturition, and the care and management of animals at different physiological stages. The advent of artificial insemination has brought in its wake the consideration of optimum semen production, its extension for use on large populations and preservation for long time use, organization of artificial insemination and frozen semen programmes, and prevention of diseases of the reproductive tract.

Though, in all reproductive programmes, the emphasis is on obtaining optimum number of calves from each animal, it is the 'milk' which comes as a byproduct that forms the base for any breeding programme. As such, another aspect of production aims at increasing the functional ability of mammary glands and hence they too need special consideration, particularly, in terms of the nutrient needs of the animal. All these aspects will receive consideration in subsequent chapters of this book.

CHAPTER TWO

Anatomy of Male and Female Reproductive Tract

The reproductive organs are composed of nontubular (testis and ovary) and tubular (fallopian tube, uterine horns, uterus, cervix, vagina vestibulum and vulva (Fig. 2.1) in females; and epididymus, vas deferens, urethra in males. According to their functions, they can be divided into primary and secondary organs of reproduction. The primary organs produce ova or sperms and hormones whereas the functions of secondary organs is to receive and to conduct both the male and female gametes nourishing and delivering the new-born.

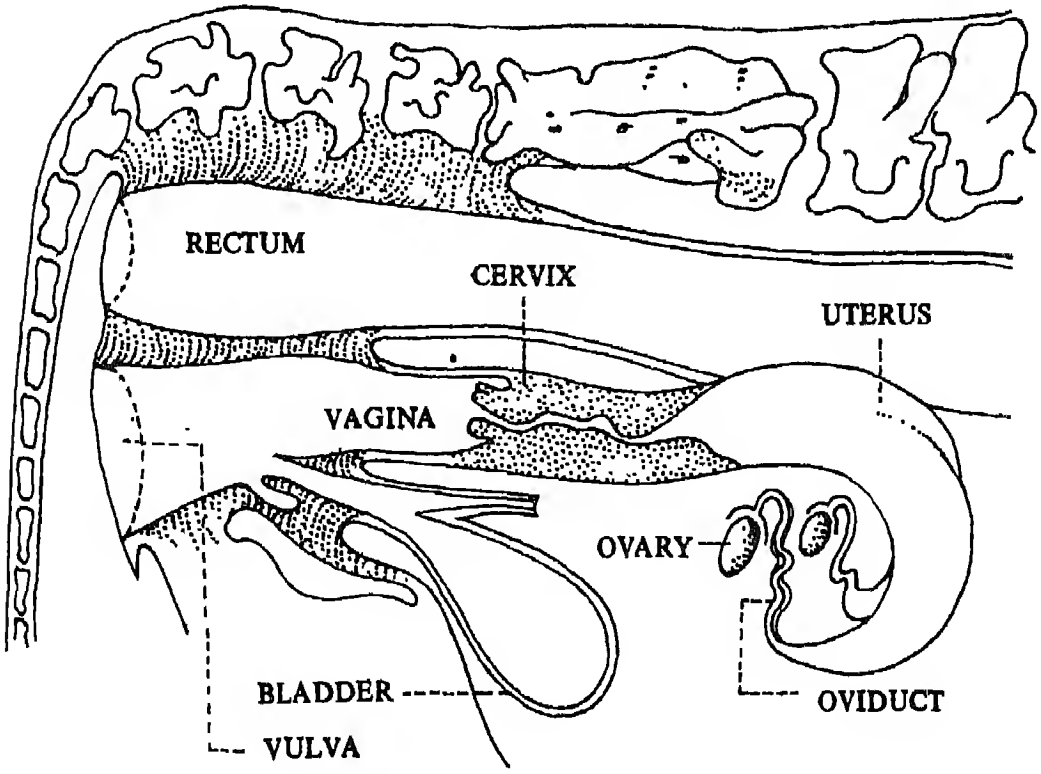

FIG. 2 1 THE REPRODUCTIVE ORGANS OF THE COW

Female Reproductive Organs:

The ovaries and the duct portion are connected with each other and are attached to the body wall by a series of ligaments—the broad ligament. This ligament consists of the mesoovarian which supports the ovary, the mesosalpinx supporting the oviduct and the mesometrium supporting the uterus The attachment of the broad ligament is dorso lateral.

Ovary The ovaries of the cow are situated near the middle of the lateral margin of pelvic inlet in front of the external iliac artery They are intrapelvic and can be palpated per rectum near the anterior extremity of uterine horns situated 40-45 cm from the vulval opening They are almond-shaped, firm, ovoid bodies, flattened on both sides. The size, form and location of the ovary varies in different animals according to breed, species, age and individual.

Each ovary is about 4 cm in length, 1.25 cm in width and its weight varies between 7 to 15 g. The right ovary is slightly larger than the left The size and weight vary according to the stage of estrous cycle, number of follicles and types of functional and regressed corpora lutea. By the onset of puberty, the ovary increases 4 to 7 times its weight at birth Ovulation occurs from any part of the exposed surface of the ovary, wherever the follicle develops.

Corporus luteum [CL]: The development of CL begins with ovulation. After ovulation, the cavity is filled with blood and lymph and there is a circumscribed area of 1 cm diameter, known as corpora haemorrhagica (CH) The term CL is applied to fully developed and regressing corpora lutea. The mature CL is primarily composed of luteal cells which are arranged in columns separated by blood vessels and connective tissue. The colour of the CL varies with the species and reproductive cycle In cattle, luteal cells contain yellow lipochrome pigment, lutein.

The maximum size of CL is attained by about 8 days (2.5-3 5 cm) after ovulation and remains unchanged until day 18 The diameter of CL at this stage is larger than that of a mature graafian follicle (GF) The CL retains its size in pregnancy. The CL of pregnancy is known as CL verum and may be larger than the CL spurium (false yellow body) of the estrous cycle

Corpora albicans: This is the spent CL after the failure of fertilization. Regression of the CL in the non-pregnant cow commences 14 to 15 days after estrus. Decrease in size proceeds rapidly and the size may be halved within 36 hours.

Crown of the CL: This is an extension of the luteal tissue in the form of prominence varying in size and width, protruding above the surface of the CL proper. Surface and consistency of CL is uniform and soft in the early developmental stage (metestrus) and subsequently there is a marked demarcation

line between the CL and the ovary proper (diestrus)

Oviduct: The oviducts are suspended in a portion of the broad ligament Oviducts are about 20-30 cm long and 1 5—3 mm in diameter in the cow. They are tortuous, wiry, hard and cartilaginous in nature. They are embedded in fat in the mesosalpinx, a portion of the broad ligament. Oviducts are difficult to palpate on rectal examination They can be subdivided into (i) infundibulum with its fimbria; (ii) ampulla and (iii) isthmus

The main function of the oviduct is to transport an egg or sperm to the site of fertilization

Uterus: This is a muscular membranous structure designed for the reception of the fertilized ovum, for the nutrition and protection of the fetus, and for the initial stage of its expulsion at parturition

In the cow, the uterus has two uterine horns lying nearly parallel to each other The body is about 2.5 to 4 0 cm long and the horns, 15-30 cm The horns are joined to each other by intercornual ligament for about half of their posterior length (caudal length).

The uterus is attached dorsolaterally by the broad ligament or the mesometrium. During pregnancy, the uterus enlarges greatly and is drawn forward and downward into the abdominal cavity. The wall of the uterus consists of (i) endometrium, (ii) myometrium and (iii) perimetrium. The endometrium is the innermost surface lined by epithelial cells and has special areas for the attachment of the placenta, called the cotyledonary areas:

The uterus serves a number of functions such as screening of spermatozoa, phagocytosin of unused sperms, capacitation, production of luteolysin substances like prostaglandins, implantation, nourishment and development of embryo and finally, it helps in the expulsion of the fetus during parturition.

Cervix. This is a powerful tubular sphincter muscle between the vagina and uterus. Its wall is harder, thicker and more rigid than the uterus In the cow, it is 5 to 10 cm in length and 2 0 to 7 0 cm in diameter and located either in the abdomen or in pelvic cavity, depending upon the parity and stage of pregnancy in animals.

The cervix is composed of 3-5 musculo fibrous annular folds and is very difficult to dilate The lumen of the cervix is lined by tall columnar epithelium Goblet cells are present in the mucosa which is so elaborately folded and branched, that it has an enormous secretory surface. The secretion is a mucus, which changes in amount and viscosity with the stages of the estrous cycle The intricate folds look like a fern leaf and give the cervix a fern like appearance

The main function of the cervix is to close the uterine lumen against microscopic and macroscopic intruders. The cervical canal is closed all the time except during estrus and parturition In pregnant animals, the cervical mucus hardens and seals off the canal by forming the

cervical plug. Breaking the cervical seal in pregnant cows usually leads to abortion or mummification of the fetus

Vagina: This is a muscular membraneous structure lying in the pelvic cavity, dorsal to the urinary bladder, and act as a copulatory organ and also as a passage for the fetus at the time of parturition. In the cow it is about 25-30 cm long and is capable of great dilation.

The wall of the vagina consists of mucosa, muscularis and serosa The mucous membrane is composed of glandless, stratified, squamous epithelial cells.

The mucus normally found in the lumen of the vagina comes largely from the cervix during estrus. The presence of a persistent hymen in the vagina may intervene in the coital function and passage of semen into the uterus through the cervix, causing hindrance to conception.

Vulva: The vulva comprises of the two labia, the dorsal and ventral commissures, the clitoris and the vestibule located between the vulva and vagina The clitoris is about 5-12 cm long in most animals, however it is practically hidden in the tissues between the vulva and the ischiatic arch

Male Reproductive Organs:

The reproductive system of males consists of paired testis, paired accessory glands and the duct system including copulatory organs (*Fig. 2.2*). A pouch of skin, the scrotum encloses the testis in the inguinal region and is responsible for the regulation of temperature, preventing damage to the spermatozoa from the environmental and body temperature effect. It is due to this thermoregulatory function of the scrotum that the testicular temperature is 5°C lower than the body temperature.

Testis The testicle of the bull is oval in shape. It measures 10-15 cm in length and 5-8.5 cm in diameter and weighs 200-500 g The long axis of the testicle is vertical.

The function of the testis is two fold (i) production of male sex hormone 'testosterone' (androgen) and (ii) production of sperms The testis also produces some amount of estrogen and inhibin from the cells of sertoli. The androgen is produced by the cells of Leydig. The sperms are produced in the seminiferous tubules, which make up over 90 percent of the testicular tissue

The Duct System:

Epididymis: The spermatozoa produced in the seminiferous tubules of the testis are carried by the collecting tubules in the testis to rete testis and various portions of epididymis namely (i) Caput (head); (ii) Corpus (body) and (iii) Cauda (tail) The length of the epididymal tube is about 30 metres

The functions of epididymis include concentration, storage, transport and maturation of spermatozoa. The two epididymis of a mature bull can accommodate upto 74.1×10^9 spermatozoa, equal to about four days production by the testis. Of the total extra gonadal sperms,

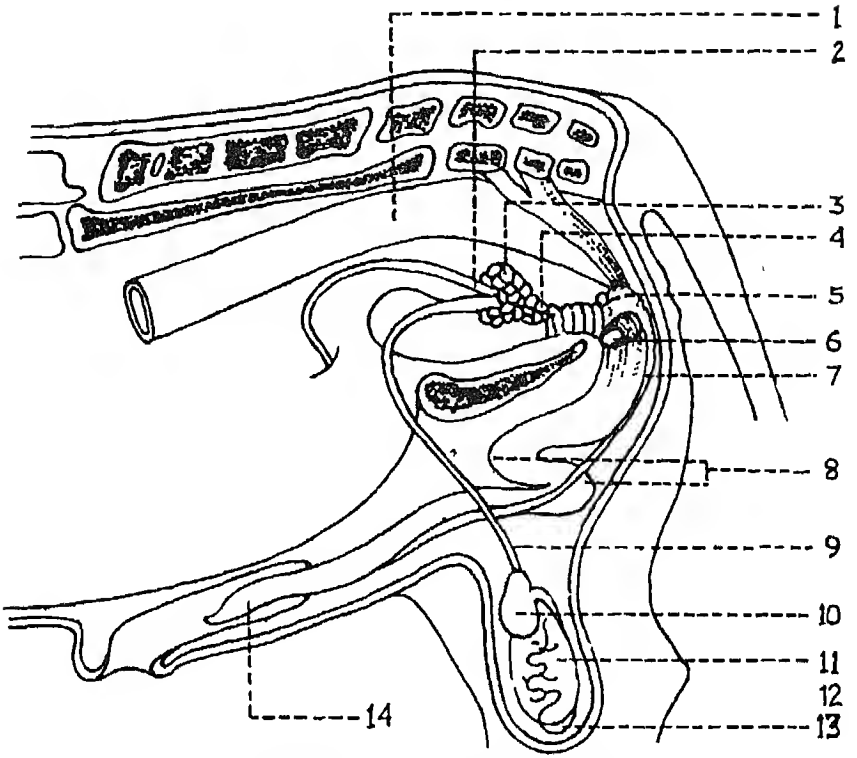

FIG. 2.2 THE ORGANS OF REPRODUCTION OF THE BULL

1. RECTUM; 2. AMPULLA, 3. VESICULAR GLAND; 4. PROSTATE GLAND, 5. BULBOURETHRAL GLAND; 6 CRUS PENIS; 7. RETRACTOR PENIS MUSCLE; 8. SIGMOID FLEXURE, 9 DUCTUS DEFERENS; 10. CAPUT EPIDIDYMIS, 11. TESTIS; 12 SCROTUM, 13 CAUDA EPIDIDYMIS, 14 FREE END OF THE PENIS

about 70 percent are stored in the cauda epididymis and about 15 percent in the caput (head).

The ductus deferens leaves the cauda epididymis and enters the pelvic at the colliculus seminalis forming distinct ampullae The ampullae have muscular walls expelling the semen from the ductus deferens into the urethra under nervous control mechanism.

Accessory Glands: The prostate, vesicular seminalis and bulbourethral glands are the three major accessory sex glands which pour their secretion into the urethra at the time of ejaculation. The secretions from them contribute to the fluid component of the semen. The function of the accessory gland secretions includes providing a liquid vehicle for the transport of spermatozoa, lubrication of the

urethral passage, maintenance of seminal pH, osmolarility and nutrition.

Seminal vesicles lie lateral to the terminal part of each ductus deferens. They are compact, lobulated and open into the urethra.

The prostate is a small gland that lies close to the urethra whereas bulbourethral glands are paired bodies lying dorsal to the urethra near the termination of its pelvic portion.

Penis: The external urethra—the penis is

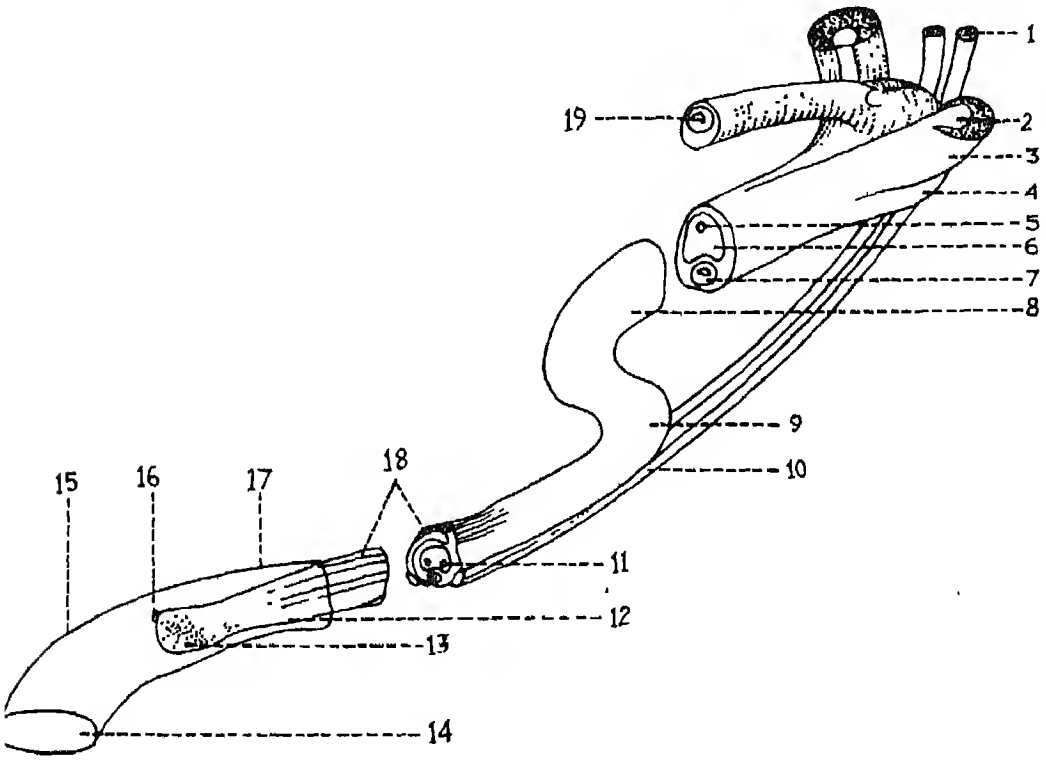

FIG. 2.3 ANATOMY OF PENIS AND PREPUCE IN THE BULL

1. LEFT RETRACTOR PENIS MUSCLE, 2. LEFT CRUS PENIS; 3 ISCHIOCAVERNOSUS MUSCLE; 4 BULBOSPONGIOSUS MUSCLE; 5. DORSAL ERECTION CANAL; 6. CORPUS CAVERNOSUM PENIS; 7 CORPUS SPONGIOSUM PENIS, 8. PROXIMAL BEND OF THE SIGMOID FLEXURE, 9. DISTAL BEND OF THE SIGMOID FLEXURE; 10 LEFT RETRACTOR PENIS MUSCLE; 11 LEFT VENTROLATERAL ERECTION CANAL, 12. FREE END OF THE PENIS; 13 CORPUS SPONGIOSUM GLANDIS; 14. ORIFICE OF THE PREPUCE, 15 PREPENILE PREPUCE; 16. URETHRAL PROCESS; 17. PENILE PREPUCE, 18. DORSAL APICAL LIGAMENT; 19 DISSEMINATE PART OF THE PROSTATE GLAND

the copulatory organ. (*Fig. 2.3*) It is capable of becoming engorged with blood.

The erection of the penis is caused by distention of the spaces with blood under nervous excitation, resulting in rigidity. A pair of smooth retractor penis muscles arise from the sactral or occygeal regions of the vertebral column. These control the effective length of the penis by their action on the sigmoid flexure ('S' shaped bent).

Prepuce: This is a fold of skin which protects the penis and is separated from glands after attaining sexual maturity under the influence of the testosterone hormone. The prepuce, besides acting as a protecting covering also, secrets a waxy material.

In the bull, the prepuce is long and narrow, 35-40 cm in length 4 cm in diameter. The preputial opening is about 5-7 cm behind the umbilicus and is 2-4 cm in diameter. The opening is surrounded by a tuft of long preputial hairs.

CHAPTER THREE

Hormones in Animal Reproduction

Hormones have been defined as specific metabolic products, produced from specific tissues and carried away from the site of production by means other than a duct, and which produce their effect on specific tissue/cells called their target tissue. There are many hormones and these have a wide range of activities. Hormones that regulate reproductive processes are derived primarily from the hypothalamus, pituitary, gonads and placenta (table 3.1)). Although all hormones are highly specific and selective in their action, a particular response to any hormone at a given time, is often modified by the presence or the absence of other hormones.

Chemically, hormones can be classified into one of several classes of substances including proteins and polypeptides, amino acid derivatives, lipids and sterols.

Protein, Polypeptides and Amino Acid derivatives:

These substances consist of amino acids linked by peptide bonds into long chain molecules. The number of constituent amino acids and, thus, the molecular weight, varies greatly from one hormone to another. Those containing fewer than a hundred amino acids are classified as polypeptides. The smaller polypeptides hormones are oxytocin, vasopressin, bradykin and insulin. Some hormones are complex proteins e g. those secreted by the anterior pituitary gland, including prolactin, follicle stimulating, luteinizing hormone, thyroid stimulating hormone and growth hormone. Some hormones like noradrenaline, adrenaline and thyroxine are amino acid derivatives.

Lipids:

One group of lipids which has been isolated from animal tissues has potent smooth muscle stimulating activity and may well be hormonal in nature. Such active lipids have been isolated from the brain, iris, intestine, lung endometrium, menstrual fluid and semen. This group of active lipids are called prostaglandins, because they were first found in semen and were thought to arise in the prostate gland. Unlike other humoral agents, prostaglandins are not localized in any particular tissue. In most instances prostaglandins appear to act locally at the site of their production. Prostaglandins exist in the form of at least six parent compounds and numerous metabolites that

exhibit a wide variety of pharmacological effects.

Sterols:

These are a very important group of lipid substances which are extracted from animal tissues using fat solvents. Unlike many lipids, they are non-saponifiable. They are crystalline cyclic alcohols occurring as free compounds or estarified with long chain fatty acids They all contain a saturated phenanthrene ring system to which is fused an additional five membered ring. Cholesterol was the first of this group of compounds to be recognized Some of the important steroid hormones are produced from Adrenals-Aldosterone and progesterone.

Study of Hormones:

The study of hormones can be carried out by the following

1. A morphological and histological identification of the tissue or organ
2. A study of the effects of removal of an organ
3. A study of the effects of replacing the removed organ by transplantation or by the injection of organ extract, or by injection of the purified hormone
4. The isolation of active principals from the organ extract and the determination of its chemical structure.

Table 3.1
Primary Hormones of Reproduction

Source	*Hormone gonadotrophin*	*Function*
Hypothalamus	releasing hormones (Gn-RH, TRH)	Causes release of FSH, LH, TSH, (from anterior pituitary)
	Somatostatin	Inhibits release of growth hormone
	Prolactin inhibiting factor (PIF)	Inhibits prolactin release
	Oxytocin (stored in posterior pituitary)	Stimulates uterine contractions, brings about parturition, sperm and egg transportations, milk ejection.
Anterior pituitary	Follicle stimulating hormone (FSH)	Stimulates follicula growth, spermatogenesis, estrogen secretion
	Luteinizing hormone (LH)	Stimulates ovulation and corpus luteum function secretion of progesterone, estrogen.

Primary Hormones of Reproduction (Cont.)

Source	*Hormone*	*Function*
	Prolactin	Promotes lactation, stimulates corpus luteum function and progesterone secretion in some species, may inhibit estrogen secretion.
	Growth hormone (GH)	Promotes tissue and bone growth
	Thyroid stimulating hormone (TSH)	Stimulates thyroxine secretion from thyroid gland
	Adrenocorticotropin (ACTH)	Stimulates adrenal cortical hormone secretion
Ovary	Estrogens	Promotes female sex behaviour, stimulates secondary sex character and growth of reproductive tract and uterine contractions, mammary duct growth, controls gonadotrophin release, stimulates calcium uptake in bones, has anabolic effects
	Progesterone	Acts synergistically with estrogen in promoting estrus behaviour and preparation of reproductive tract for implantation, stimulates endometrial secretions, maintains pregnancy, stimulates mammary alveolar growth, control gonadotrophin secretion
	Relaxin	Loosening of symphysis pubies and sactroshiatic ligaments Opens birth canal
	Inhibin	Inhibis FSH release

(Cont)

1	2	3
Testis	Androgens	Develops and maintains accessory sex glands, stimulates secondary sexual characteristics, sexual behaviour, spermatogenesis has anabolic effects
	Inhibin	Inhibits FSH release, regulates gametogenesis.
Placenta	Human chononic gonadotrophin	Similar to LH activity
	Pregnant mare serum gonadotrophin	Similar to FSH activity
	Placental lactogen	Has GH activity
	Placental luteotropin	Maintains CL
	Estrogens and progesterone	Same as mentioned above.

Neuroendocrinology:

Neuroendocrinology involves the interactions of the nervous system with endocrine glands. Three types of cells mediate communication between organs, neurons, neuroendocrine cells and endocrine cells. The neuro-endocrine cell converts a neuronal input to an endocrine output. The neuron releases a neurohumor that diffuses for only a short distance. Neuroendocrine reactions play a major role in regulation of reproductive, metabolic and behavioural functions of the body.

Besides the neurohumoral control of the anterior pituitary through release and inhibitory hormones, the hypothalamus directly produces the post-pituitary hormones, vasopressin (Antidiuretic hormone-ADH) and oxytocin. Vasopressin acts upon the epithelial cells of the distal portion of the renal tubule to cause reabsorption of water. Oxytocin causes contractions of uterine muscles. It also causes increased frequency of contraction in the oviduct and, thus, is involved in the transport of both male and female gametes. Estrogen enhances the responsiveness of smooth muscles to oxytocin. Milk let-down or milk ejection reflex is an example of neuroendocrine

reflex. The lactating animal becomes conditioned to visual and tactile stimuli associated with suckling or milking or the sound of utensils or calf calls. This conditioning induces the release of oxytocin into the circulation. Oxytocin then acts on the myoepithelial cells (smooth muscle cells) that surround the alveoli in the mammary gland The contraction of myoepithelial cells puts pressure on the alveoli, which displaces milk into the duct system of the mammary gland, resulting in milk let-down. This hormone is used clinically for milk let-down and expulsion of retained placenta. This hormone is also important for parturition. Summary of neurohormones involved in reproduction is given in table 3.2

Table 3.2

Summary of neurohormones involved in reproduction is given in table 3.2

Gland	*Hormone*	*Functions*
Hypothalamus	Prolactin inhibiting factor (PIF)	Inhibits prolactin release
	Prolactin releasing factor (PRF)	Stimulates prolactin release
Anterior Hypothalamic area	Luterinizing hormone releasing hormone (LHRH)	Stimulates surge of LH and PSH release.
	Gonadotrophin releasing hormone (Gn RH)	
Ventromedial nucleus	Luteinizing hormone releasing hormone (LH-RH)	Stimulates tonic release of LH and FSH
Dorsomedial area	Thyrotropin releasing hormone (TRH)	Stimulates release of TSH and prolactin
Paraventricular and Supraoptic area	Oxytocin	Uterine contractions, milk let-down, gamete transport

Placental Hormones

Placental hormones include pregnant mare serum gonadotrophin, human chorionic gonadotrophin and placental lactogens.

Pregnant mare serum gonadotrophin (PMSG) is a glycoprotein hormone with a high content of sialic acid. This gonadotrophin is secreted by endometrial cups in the estrus. This uterine secretion forms from the sixth week of pregnancy and persists until about the twentieth week of gestation. PMSG displays both FSH-like and LH-like activity, though FSH function is predominant. PMSG has frequently been used to promote extensive follicular development prior to super ovulation for embryo transfers. It has also been used to promote follicular development during the estrous period in females.

Human chorionic gonadotrophin (HCG) is a gonadotrophin excreted in the urine of pregnant women. It is synthesized by the syncytiotrophoblastic cells of the placenta. HCG converts the corpus luteum of the menstrual cycle to the corpus luteum of pregnancy. HCG has predominant LH-like functions.

Placental lactogenic (PL) hormones have chemical and biological properties similar to those of both GH and prolactin and they are excreted by the placental tissue. The concentrations of ovine PL in maternal serum are low during the first two trimesters of pregnancy and rise dramatically during the last trimester. Placental lactogens may be important regulators of maternal metabolism to ensure the availability of adequate nutrients to the developing fetus.

Besides the above hormones, there are other hormones whose roles, though important, are secondary to the hormones discussed above. The hormones along with their functions are listed in the table 3.3

Table 3.3

The Secondary Hormones of Reproduction

Source	*Hormones*	*Function*
Anterior pituitary	Somatotrophis (STH)	Body growth, protein synthesis
	Thyrotropin (TSH)	Stimulation of thyroid gland, thyroxine release and iodide uptake by thyroid
	Adrenocorticotropin (ACTH)	Stimulation of adrenal cortex, release of adrenal corticoids.

(Cont.)

1	2	3
Posterior pituitary.	Vaso Pressin (ADH)	Water balance
Thyroid	Thyroxine	Body growth, development, maturation
	Tri-iodo thyronine	Oxidation of feed
	Thyro-calcitonin	Calcium metabolism
Adrenal Cortex	Aldosterone	Electrolyte and water metabolism
	17-OH corticoids	Carbohydrate, protein and fat metabolism.
	(Cortisol, cortisone cortico-sterone).	
Pancreas	Insulin	Carbohydrate, fat and protein metabolism.
Parathyroid	Parathormone	Calcium and phosphorous metabolism

Hormones in Estrous Cycle:

There are three major events which occur during an estrous cycle.

1. Endocrinological events
2. Morphological events
3. Behavioural events

Each of these changes are to occur independently but must be synchronous with each other In case they occur out of phase, then the reproductive process may lead to failure.

The estrous cycle is controlled by the interaction of FSH, LH, estrogen and progesterone The secretory patterns and their relative effects vary among different species which leads to variations in length of luteal and follicular phases of the cycle as well as to differences in duration of estrus.

The follicular phase of the cycle is characterized by rapidly decreasing levels of progesterone and a peak in blood

levels of estrogen. This decline in the level of progesterone followed by the rapid rise in estrogen is an essential requirement for the onset of behavioural estrus. Blood levels of LH increase approximately twenty-fold towards the end of the follicular phase. This may result from removal of the negative feedback influence of progesterone. The peak of estrogen during the follicular phase exerts a positive feedback influence on the hypothalamo-hypophyseal axis, resulting in the ovulatory surge of LH, which occurs approximately 12 hours after the onset of estrus. Peak levels of FSH and prolactin occur simultaneously with the ovulatory surge of LH. The increase in LH and FSH is caused by hypothalamic release of Gn RH, whereas prolactin secretion is due to inhibition of PIF by the preceding peak of estrogen.

Ovulation does not appear to be a mechanical process of rupture due to excessive internal pressure but a proteolytic enzyme degradation process. Besides this proteolytic activity, the ovulatory surge of LH also increases follicular synthesis of prostaglandins (PGE_2)

Following ovulation, the wall of the follicle gradually thickens due to hypertrophy and hyperplasia of granulosa cells. These cells rapidly proliferate, fill the cavity and begin to secrete progesterone. This structure, called corpus luteum, continues to increase in size and weight and obtains full growth and function. Seven to nine days after ovulation, the size of CL is highly correlated with its ability to secrete progesterone. A complex of hormones rather than one hormone is important for the growth of CL.

After about 16-18 days of the cycle, depending upon whether fertilization has occured or not, the CL begins to regress. A luteolytic substance produced from the uterus passes from the uterus to the ovary. This luteolytic substance is prostaglandin F_2 alpha.

CHAPTER FOUR

Gametogenesis

Gametogenesis refers to the formation of the male and female gametes; the spermatozoa and oocyte, respectively.

Spermatogenesis

The process of formation of male gamete, i.e. spermatozoa, is called spermatogenesis. Spermatozoa are highly specialized cells and are formed within the seminiferous tubules of the testis, after a complex series of developmental changes of the primordial germ cells. Once fully formed and ejaculated as a cellular suspension in the form of semen, this cell is incapable of further growth

The spermatozoa originate from the epithelium of the seminiferous tubules. The germ cells called spermatogonia, undergo a continuous series of cellular divisions and developmental changes, beginning at the periphery of the tubule. These cells devide several times to produce A_1, A_2, A_3 and B type of spermatogonia. The 'B' type spermatogonia (2n) divide to form primary spermatocytes (2n) which further divide by meiosis to form secondary spermatocytes (n). This cellular division reduces the DNA content of the cells to one half that of the somatic cells. Such haploid cells, resulting from the divisional process, the secondary spermatocytes, give rise to spermatids. The entire divisional process from spermatogonia to spermatid stage takes approximately 45 days in the bull and is called the process of spermatocytogenesis.

The divisional process can be summarized as:

Primordial germ cells-spermatogonia types A and B—

Primary spermatocytes–Secondary spermatocytes—

Spermatids-Spermatozoa (*Fig 4.1*).

The modification of the spermatids continues through a series of morphological changes. These changes consist of.

1. Condensation of the nuclear chromatin in the sperm head.
2. Formation of the sperm tail or flagellar apparatus.
3. Development of the acrosomal cap from golgi apparatus.
4. Congregation of mitochondria to form the mitochondrial level of the mid piece.
5. Development of centrioles into fibrils.

As a result of this metamorphosis, a fully formed, though immature sperma-

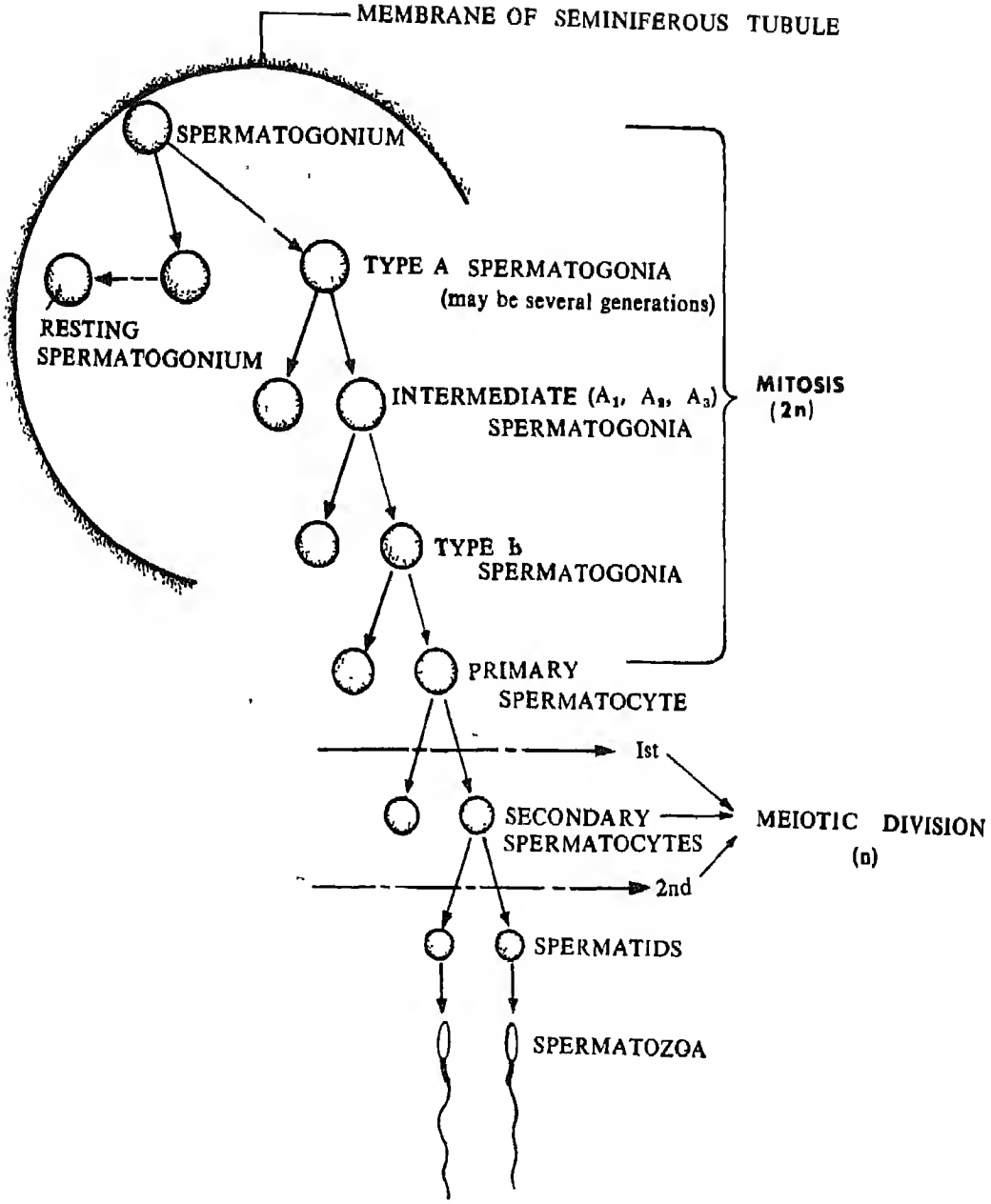

FIG 4.1 THE PROCESS OF SPERMATOGENESIS

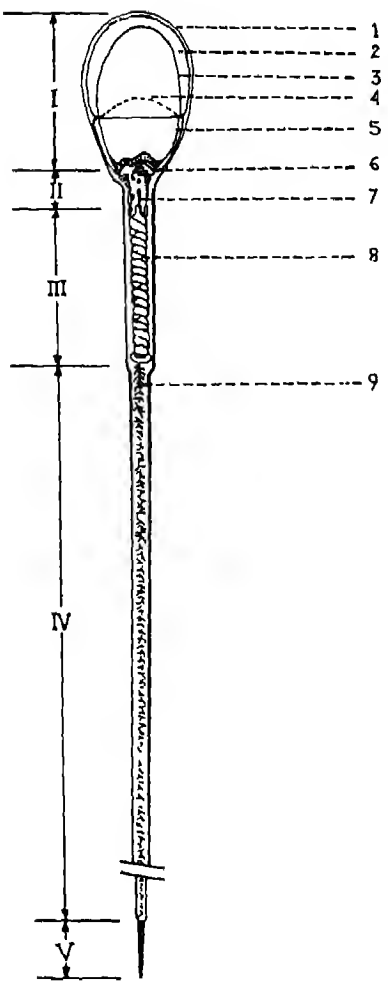

FIG. 4.2 SKETCH OF A BOVINE SPERMATOZOON

I. HEAD; II NECK; III. MIDDLEPIECE, IV PRINCIPAL PIECE; V. END-PIECE
1. CYTOPLASMIC MEMBRANE.
2. ACROSOME, 3. NUCLEAR MEMBRANE;
4. NUCLEUS, 5. POSTNUCLEAR CAP,
6. PROXIMAL CENTRIOLE; 7. AXIAL FILAMENT; 8 . MITOCHONDRIAL HELIX; 9. FIBROUS SHEATH.

tozoa is produced. The metamorphic changes which the spermatid undergoes is described as spermiogenesis

The process of spermatogenesis is continuous and repeats itself indefinitely

Structure of the Spermatozoa:

The fully formed spermatozoa are elongated cells consisting of a head, neck, *middle piece* and *tail* (*Fig 4 2*). The head of the spermatozoa is covered by a thin double walled structure called the acrosome or acrosomal cap The neck is short and connects the sperm head with its flagellum or tail The anterior portion of the flagellum is wrapped by the mitochondria in the form of a helix The spermatozoa, thus, has a streamlined structure The whole cell, all along its length, is covered by a thin protecting lipoprotein sheath. Spermatozoa of different species differ in their size and shape but all have the above described essential structures.

The sperm head is oval, flattened, containing the highly compact chromatin, which comprises almost entirely of DNA. The chromosome number and, hence, the DNA content of the sperm nucleus, is half that of the somatic cells of the same species. The spermatozoa are of two types as far as sex chromosomes are concerned. Gametes carrying 'X' chromosome give rise to female embryos, whereas those carrying 'Y' chromosomes give rise to male embryos. The head is covered by acrosome which contains specific enzymes involved in the fertilization process.

The neck or connecting piece forms a basal plate and articulates with a depression into the posterior surface of the nucleus (head). The basal plate of the neck is continuous posteriorly with the nine pairs of coarse fibrils that project posteriorly throughout most of the tail. The neck is slender and can be easily fractured if the sperm is not handled properly.

The entire middle piece is full of mitochondrial sheath which is arranged in a helical pattern around the longitudinal fibrils of the tail and is believed to generate energy required for sperm motility.

The middle piece and the rest of the tail is composed of nine pairs of fibrils or microtubules that are arranged radially around two central fibrils. The end piece contains only the central fibrils covered by plasma membrane

Ovagenesis

Ovagenesis or ovigenesis is the process of formation of an egg. This process starts very early in life from the germinal epithelium in the ovary The germinal epithelium cells sink into the ovarian cortex where they multiply and grow. An egg (ovum) is a highly differential cell that is capable of being fertilized and subsequently undergoes embryonic development. Mammalian eggs were first recognized by de Graafin in 1672 and then described and identified by Cruickshank in 1797 and von Baer in 1827. The following discussion is concerned with the formation, structure, ultrastructure, biochemistry, transport and manipulation of mammalian egg.

The precursor cells of either male or female gametes, called gonocytes, originate probably from extra-embryonic endodermal tissue (extragonadally). They migrate to the presumptive intra-embyonic gonadal zone where they differentiate either into oogonia or spermatogonia. In the female fetus, the germinal epithelium forms into clusters in which one gonocyte differentiates into an oogonia containing typical cell constituents, e g. golgi apparatus, mitochondria, nucleus and one or more nucleoli. The oogonia then undergo proliferation prior to or shortly after birth, resulting in the ovaries containing the sole reservoir of all future ova or oocytes. Thereby (i) the enlargement of the cytoplasm by accumulation of different sizes of granules of deutoplasm (yolk); (ii) the development of an egg membrane (zona pellucida); and (iii) the mitotic proliferation of follicular epithelium and adjacent tissue takes place These follicular cells may serve as nurse cells by providing the deutoplasm of the oocyte. By maturity, the egg has accumulated reserves of maternal to provide an energy source for subsequent development. Factors determining which of the ovarian oocytes are destined to begin their growth or to complete their growth during a productive cycle, are unknown.

There are two phases in the growth of the follicle. During the first phase, growth is rapid and intimately associated

with the development of the ovarian follicle. Attainment of its mature size occurs at about the time antrum formation begins in the follicle. During the second phase, the oocyte does not grow in size, while the ovarian follicles responding to pituitary hormones increased very rapidly in diameter This growth is, in fact, confined primarily to follicles in which the egg has attained its full dimensions.

During the latter phase of follicular growth, the oocyte undergoes maturation. The nucleus which has entered into the prophase of the meiotic division during the growth of the oocyte, prepares to undergo reduction division. The nucleoli and the nuclear membrane disappear and the chromosomes condense in a compact form. The centrosome then divide into two centriols around which asters appear (groups of radiations at both poles of the oocyte). These asters move apart and a spindle is formed between them. The chromosomes in diploid pairs are set free in the cytoplasm and become arranged on the equatorial plate of the spindle (metaphase I). The primary oocyte now undergoes two meiotic division. In the first division, two daughter cells arise, each containing one half of the chromosome complement (2n). However, unlike the divisions occuring in spermatogenesis, one acquires almost all of the secondary oocyte, the other much smaller cell, is known as the first polar body. At the second maturation division, the secondary oocyte divides into the ootid (n) and a second polar body (n) The two polar bodies, containing very little cytoplasm are entrapped within the zona pellucida of the oocyte, and here they degenerate. The first polar body may also divide; thus, the zona pellucida may contain one, two or three polar bodies.

It should be pointed out that it is the secondary oocyte which is liberated at ovulation (primary oocyte in the case of the horse). The oocyte continues the process of maturation until fertilization when it becomes a 'zygote'. In the process of oogenesis, one primary oocyte gives rise to an egg, in spermatogenesis, one primary spermatocyte gives rise to four sperms

CHAPTER FIVE

Composition of Semen and Factors Affecting Its Quality and Quantity

The fully developed spermatozoa, after it is released in the lumen of the seminiferous tubule, is transported from the testis to the epididymis. In the epididymis it is stored for a while, undergoes further maturation, until it is mixed at ejaculation, with the secretions of the male accessory sex glands, to form semen. Spermatozoa are produced continuously in the testis of a healthy, mature, male in billions The volume of semen produced in different species differs.

Semen Composition:

Sperm cells are suspended in a liquid medium known as seminal plasma Sperms are produced in the testis. The plasma is mainly the secretions of the male accessory sex glands. The volume and composition of semen differs in different species as it depends largely upon the seminal plasma, which contributes to the bulk of semen.

Semen plasma is a buffered medium containing either a source of energy directly available to sperm (like fructose, sorbitol), or one that can be used as energy substrate to be of use later, in the female genital tract.

Seminal plasma has pH of about 7.0 and osmotic pressure similar to blood (i.e. equivalent to 0.9 percent sodium chloride).

Sodium and potassium are predominantly present in the seminal plasma of the bull (Table 5.1) Concentrations of calcium and magnesium are at a low level. The concentration of potassium is greater in the sperm than in the seminal plasma, while the reverse is true for sodium Potassium is responsible for the viability of the sperms. Semen also contains citrate and bicarbonate buffers. Large amounts of lactic acid are formed by the sperm from utilization of fructose in the semen.

There are organic compounds which are present in the seminal plasma in appreciable quantities. These compounds are fructose, citric acid, sorbitol, inositol, glycerylphosphorylcholine (GPC), ergothioneine and they are not found elsewhere in the body in such high concentrations Most of these compounds originate from the male acces-

TABLE 5.1

Characteristics and Approximate Chemical Composition of Seminal Plasma

Constituent or Property	Bull
Volume of ejaculate (ml)	5—8
pH	*6 9(6.4—7 8)
Sodium	225±13
Potassium	155±6
Calcium	40±2
Magnesium	8± 3
Chloride	174—320
Fructose	460—600 (SV)
Sorbitol	*(10—140) (SV)
Citric acid	620—806 (SV)
Inositol	*35 (25—46)
Glycerylphosphorylcholine (GPC)	*350 (100—500) (E)
Ergothioneine	*0
Protein (g/100 ml)	*6 8

* Analyses on whole semen Mean values (mg/100 ml of seminal plasma unless otherwise indicated) are given

SV, mainly from seminal vesicles: E, mainly from epididymis.

sory sex glands and are indices of the well functioning of these glands.

Mucoproteins, peptides, free amino acids, lipids, fatty acids, vitamins and a variety of enzymes may also be present in the seminal plasma of some species.

Factors Affecting Semen Quality and Quantity:

There are several factors which affect the quality and quantity of semen produced by an animal. These factors may affect semen *in vivo* (during production) or *in vitro* (after it has been ejaculated). Amongst the factors which affect the semen production *in vivo*, the atmospheric temperature and nutrition are the most important.

The atmospheric temperatures, which can raise the body temperature of the males considerably, for prolonged durations, could be detrimental to sperm production as spermatogenesis is affected. Amelioration in microclimate, i.e., where the temperature has been brought down artificially, has been reported to be beneficial.

Nutrition directly affects semen production in males. Above average nutrition levels have to be maintained for study bulls. Bad nutrition adversely affects semen production. That is why there is a necessity of providing better nutrition to study animals. Vitamins, particularly Vitamin 'A' has a profound effect on semen production.

Younger animals tend to give better quality and quantity of semen. Semen characteristics and sperm output of normal mature dairy cattle is given in *Table 5.2.*

There are several factors which affect semen quality *in vitro*, that is, after it has been ejaculated by the male. In case collection of semen is done in the artificial vagina, a thorough sterilization of the equipment and the materials to be used is of great importance Otherwise, survival of good quality semen will be adversely affected

TABLE 5.2

Semen characteristics and Sperm Output of Normal Mature Dairy Cattle

Item	Dairy Cattle
Number of semen collections per week	2-6
Volume(ml)/ejaculate	5-10
Sperm concentration/ ml (million)	1000-2000
Total Sperm/ejaculate (billion)	5-15
Total Sperm/week (billion)	15-40
Progressively motile sperm (%)	50-75
Morphologically normal sperm (%)	70-95

The media in which the semen is to be preserved should have about the same tonicity as semen or blood. Sperms are most active and survive for the longest period at a pH of about 7 0. Sperms are fairly rapidly immobilized by acid conditions but their vitality can be restored in some species, if the pH is brought back to neutral promptly. The motility and metabolism of sperms can be reversibally suppressed by high concentrations of carbon dioxide or with the incorporation of a particular concentration of fatty acids in the buffer media, for prolonged periods, without great loss of fertility

Moderate dilution of semen stimulates activity and increases the life span of spermatozoa. Excessive dilution however, even in an optimal medium, depresses motility A protective agent like egg yolk could help in increasing the dilution.

Semen quality is greatly influenced by ambient temperature. That is why semen is examined in laboratory conditions at 37°C which is close to body temperature An increase in this temperature will excite the sperms, enhance their metabolism and reduce their life span. Above 50°C, sperm suffer an irreversible loss of motility At body temperature, the life span of spermatozoa in neat semen is only a few hours. Sperms are prone to sudden cold shock. A gradual decrease of temperature for preservation is essential for maintaining the integrity of spermatozoa. It is possible to preserve semen at temperatures near freezing point or below it for several days.

CHAPTER SIX

Puberty and Estrous Cycle in Bovines

Puberty is the period of maturity when the dairy animals, for the first time, are able to release gametes. Among females it is characterized by the initiation of the estrous cycle. In males, the animal shows a sound desire for mating and is capable of donating semen. Puberty in dairy animals is a much more dramatic event than in other animals. These changes are due to physiological and anatomical alterations that occur at the cellular level under the influence of reproductive and metabolic hormones. These changes go side by side and it is not possible to seperate the individual events responsible for the onset of puberty in dairy animals. In addition to the externally visible manifestation of sexual receptivity among females, certain changes in the ovarian, uterine and vaginal histology make it possible to follow the pubertal events.

The event of puberty is generally influenced by physiological, environmental and management factors. Different breeds show puberty at different ages. Nutrition plays an important role in the age at puberty. Poor nutrition delays it. Though the ovary is capable of ovulation at puberty, the formation of the ovum occurs much earlier in life, during the embryonic and prenatal period.

After the event of puberty, the animal exhibits a periodic expression of cyclic reproductive behaviour, known as the estrous cycle. This cycle lasts from 18 to 24 days with an average length of 21 days among dairy animals (cattle and buffaloes). The duration of the estrous cycle varies among breeds and even among animals of the same breed. It is also influenced by age, season, climate, nutrition and disease status of the animal.

The estrous cycle is divided into two major unequal phases; (i) follicular phase, characterized by rapid development of the follicle simultaneous to rapid regression of previous cycle corpus luteum and ends with estrus or ovulation of one or more graafian follicle, and (ii) luteal phase, characterized by the formation of corpus luteum after ovulation. The major phases of the reproductive cycle are:

Proestrus	3 days
Estrus (heat)	18-26 hours
Metestrus	3-4 days
Diestrus	12-16 days

Proestrus:

In the proestrus phase, under the influence of the hypothalamic and pituitary hormones, the follicles are stimulated through quick growth to maturation and development of a mature graafian follicle This is due to lowering of progesterone levels after the lysis of the corpus luteum which occurs during this phase. This mature graafian follicle is capable of producing a steroid hormone (estrogen) which stimulates the psychic behaviour of the female and brings it to estrus.

Estrus:

Estrus is a fairly well defined period characterized by sexual desire and the acceptance of the male by the female domestic animal. It coincides, as described earlier, with the maturation of an ovum in the ovary under the influence of the follicle stimulating hormone, released from the pituitary, and estrogen, secreted by the ovary The period lasts for about 10-24 hours on an average but can be as short as 4-6 hours or extend up to 36 hours in some animals Usually, multiparous animals exhibit weak symptoms and parous animals, pronounced (strong heat) symptoms. The estrus period can be divided into (i) early estrus, (ii) mid estrus, and (iii) late estrus, depending upon the nature of heat symptoms exhibited by the animals.

External Estrus Symptoms:

The external heat symptoms are characterized by a peculiar systemic excitement that usually lasts for a definite period and then passes off. Females of each species behave slightly differently but their behaviour has, in each case, the following points of similarity·

1. The genital organs, specially the vulva and vagina, become congested, swollen and there is a discharge of mucus from the vulva.
2. The nature of mucus discharge depends upon the stage of estrus During mid-estrus, the discharge changes to copious and watery and a dried smear will give a fern leaf pattern when examined under a microscope. The discharge becomes viscid or turbid during late estrus
3. The animal is excited, raises its head and tail and shows alert posture. Its appetite become irregular, and milk yield gets reduced.
4. The animal becomes restless and shows a liking for the opposite sex. Licking of the genetalia of other animals is also indicative of estrus behaviour.
5. Animals in estrus usually mount other animals and also stand to be mounted (standing heat) Standing heat is the single most important sign of estrus behaviour.

6. The animals start bellowing and urinate frequently.

Internal Estrus Symptoms:

1. Increase in turgidity and tone of uterus.
2. Cervix relaxed and os-uterus patent.
3. Mature graafian follicle palpable in the ovary.

Detection of Estrus:

Accurate and timely detection of estrus is probably the most important event for enhancing reproductive efficiency of animals. Therefore, the animal husbandry man engaged in the field of artificial insemination often needs to be trained to identify animals in estrus, so that these may be inseminated at the right time. The signs and degrees of external manifestation of proestrus, estrus and postestrus should be thoroughly understood. The female animals can be spotted for estrus behaviour by adopting the following management practices

1. The pubertal heifers and postpartum animals should be kept separately from young and old noncyclic animals.
2. Cows to be observed for estrus should be grouped and watched carefully for 20 to 30 minutes or more, 2-3 times every day.
3. The use of heat expectancy charts is very helpful if records of previous estrus periods are accurately recorded.
4. The external symptoms such as mucus discharge from vulva, bellowing, frequent micturition and jumping or mounting on another animals should be taken as positive estrus symptoms for heat detection. Standing heat should be taken as the right time for insemination.
5. The help of teaser bulls or breeding bulls can be used for detecting the accurate stage of estrus exhibition The teaser bulls can be paraded two to four times in a day or can be kept along with the females in the herd
6. Frequent rectal and vaginal examination of the genital tract will determine the proper time to inseminate cycling cows and buffaloes with silent and even unobserved estrus.
7. Estimation of hormonal profiles such as progesterone gives an indication of the stage of estrus in animals.
8. A number of estrus detection aids like heat mount detection (KAMAR), chin ball marker, pedometer, estroscope, mucus conductivity meter have been employed with success.
9. Recently trained dogs have been employed for picking up

females in heat; though the bull is the best detector of estrus, there is no substitute for an efficient human eye

Metestrus:

This period follows estrus and is characterized by cessation of estrus symptoms. It has a duration of 3-4 days. During this phase, the female ovulates. Theca interna and granulosa cells of the ovary start multiplying at a fast rate and develop into a special endocrine tissue, the corpus luteum, which produces progesterone.

Ovulation:

Ovulation is described as the process of shedding of ovum by the ovary. At ovulation, the egg is released, while embedded in a solid mass of follicular cells, the cumulus oophorus, which protrudes into the fluid-filled antrum. The release of ovum occurs at the apex of the follicle. Gonadotrophin stimulation rapidly increases the synthesis of hormones and accelerates steroidogenic action. In the theca interna, the rising level of steroids, particularly estrogens in the follicle feed back on the pituitary gland and cause the inhibition of further production of follicle stimulating hormone (FSH) and release of large quantities of luteinizing hormone (LH) This surge of LH causes the process of ovulation to occur.

Corpus Luteum:

The corpus luteum (plural, corpora lutea) is a temporary endocrine organ which functions for only a few days in the cycling non-pregnant animal but functions through most of the pregnancy in other domestic species. Immediately after ovulation, there is enough hemorrhage into the follicular cavity, especially in the cow, for a blood clot to develop. The blood-filled follicle, now devoid of the ovum, is commonly referred to as a corpus hemorrhagicum. This clot of blood serves as a physical framework and a nutrient medium for the quick proliferation of the granulosa and theca cells which are mainly responsible for the rapid development of this endocrine organ.

The growth of the luteal cells is one of the fastest known in biology. Within 3 to 4 days, the blood clot has been adequately invaded by the new luteal cells so that the blood-filled cavity loses its dark colouration.

Following ovulation, the granulosa cells, which remain intact, begin to hypertrophy and take on lipid material and become the primary lutein cells of mature corpora lutea Next, capillaries invade the granulosa lutein cells from the surrounding theca interna and carry some lipid-containing theca interna cells with them. These theca lutein cells become dispersed among the granulosa lutein cells in most species

The corpus luteum is one of the most vascular organs of the body. Columns of lutein cells are separated by vessels which must nourish this new organ because of

its metabolic activity (steroid synthesis). Vascular growth and lutein cell differentiation are complete by day 7 of a 20-day cycle. Full size is usually attained by day 8 or 9 of the cycle. The newly formed organ is termed a corpus luteum verum if the animal becomes pregnant and the corpus continues to function. In the cycling non-pregnant animal, the newly formed organ is termed a corpus luteum spurium, since it is destined to early failure. The corpus luteum verum may increase slightly in size until mid-pregnancy

There is microscopic, then gross, evidence of regression of corpora lutea by days 15 to 16 in a 20-day infertile cycle. Physiological function abruptly ceases, but the gross physical appearance of the organ continues for several days or weeks in most animals. The granulosa lutein cells degenerate rapidly showing cytoplasmic vacuolation and pycnotic nuclei. Progesterone production plummets sharply, even more dramatically than anatomical changes Corpora lutea then decrease in size along with degeneration of capillaries. Gradually, the granulosa lutein cells are replaced by fibroblasts, and the other cells become enmeshed in the forming connective tissue. The degenerating avascular non-functional corpus is termed a corpus luteum albicans (white) or simply a corpus albicans Slow physical degeneration occurs, usually taking 2 to 3 weeks. For several additional estrous cycles, a visible connective tissue scar remains on the ovary.

After ovulation in the cow, granulosa cells undergo hypertrophy and become filled with droplets of a yellow lipid material as the corpus luteum forms. The maximum size of the bovine corpus luteum is attained by day 16 then it degenerates rapidly. The bovine corpus luteum changes from a light brown to gold by day 7, then to a golden yellow by day 14. Between days 14 and 20, the corpus progressively changes from yellow to orange to brick-red. The brick-red colour remains for several cycles as the old corpus gradually regresses The corpora lutea of the cow is dark because of a yellow pigment, lutein.

CHAPTER SEVEN

Fertilization, Embryogenesis and Gestation in Dairy Animals

The fusion of two cells, the male and female gametes to form a single cell, the zygote, is known as fertilization. Hence fertilization is a dual process:

1. **Embryological aspect:** This involves the activation of the ovum by the sperm and, thus, leads to fertilization and development of the embryo
2. **Genetical aspect:** Fertilization involves introduction into the ovum of the hereditary material from the sire The hereditary material is the chromosomal DNA in the sperm nucleus

In most mammals, fertilization occurs after the first polar body has been extruded so that sperms penetrate the ovum while the second reduction division is in progress

Site of Fertilization:

This is the lower portion of the ampulla of the oviduct after 1-2 hours of ovulation.

The sperms undergo some changes-capacitation before they can activate the ova. The size of ampulla, number of sperms, the rate of swimming, surface area of the ovum, are responsible for the ovum-sperm meet. Fertilization is an entirely random process and there are equal chances of any sperm fertilizing any ovum The sperms from different males may differ in their fertilizing capacity

The sperm has to penetrate the following barriers before entering into the ovum This prevents polyspermy.

1 The cumulus mass,
2. Zona pellucida,
3. Vitelline membrane.

Nidation:

The embryo is said to be implanted or attached when it becomes fixed in position and a physical contact with the maternal endometrium is established In implantation, the embryo becomes attached to the wall of the uterus. In farm animals, the embryo remains in the uterine cavity Movement of the blastocyst within the uterus becomes increasingly restricted as it expands. Implantation takes place 20-40 days past coitum in cows In the cow the embryo implants to the distal part of the horn, adjacent to the

ovulatory ovary. The position in which the blastocyst implant, is fixed and is determined by the relationship between the blastocyst and uterine wall, rather than by the action on the blastocyst of any external factor such as gravity.

Gastrulation:

This is a stage of embryonic development occuring in various stages. Gastrulation consists of movements of cells or groups of cells in such a way as (i) to convert the embryo from a two-layered into a three-layered structure (ii) to bring the future organ forming organs into their definitive positions in the embryo.

In mammals, gastrulation involves the cells of the embryonic disc only. From the embryonic disc, three types of tissue differentiation takes place (a) endoderm (b) mesoderm and (c) ectoderm.

The fetal tissue is developed from these three layers and converted into the respective organs as under

Three germinal layers:

Endoderm	Mesoderm	Ectoderm
Primitive gut	Head	Skin
Respiratory system	Skull	Brain
Digestive system	Skeleton	Spinal cord
Liver	Muscles	Sensory organs
Pancreas	Kidney	Mammary glands
	Heart	
	Reproductive tract	
	Connective tissue	
	Urinogenital organs	
	Circulatory system.	

Gestation:

The duration of gestation is genetically determined although it can be modified by maternal, fetal, hormonal and environmental factors. The gestation period in cows and buffaloes is 275-280 and 300-310 days, respectively. Size and characteristics of the bovine fetus during pregnancy is given in table 8.1.

Placenta:

The placenta is a fusion of the fetal membranes to the endometrium to permit physiological exchange between fetus and mother. The mother not only serves as a source of nutrition but also shares in respiration and excretion. The placenta functions as a nutritive, excretory, endocrine and protective organ. It is inti-

mately united with the maternal tissue and is not rejected until parturition. During the early stages of gestation, the placenta increases in size through the active proliferation of the trophoblastic cells. During the mid-gestation period, it attains a near maximum size. The extra embryonic membranes are differentiated into amniotic, allantoic and chorionic (serosa). The amnion surrounds the fetus. The serosa is the outermost membrane and is in direct contact with the endometrium. The allantois is located in between chorion and amnion, continues with the anterior extremity of the bladder by the way of the umblicus passing through the umblical cord. The inner layer of the allantois is fused with the amnion, the outer layer with the serosa forming the chorioallantois. In the case of cows the placenta is of the Epitheliochorial type and cotyledonary in shape with five cell layers.

The number of functional caruncles increases as pregnancy progress. In cattle, placentomes start to form at about 4 to 5 weeks immediately around the fetus and progress towards the distal limit of the chorioallantois in the non-gravid horn (12-13 weeks). During pregnancy, they also enlarge their original diameter. Those situated in the middle of the gravid horn develop to a larger size than those at the extremities.

Table 8.1

Size and Characteristics of the Bovine Fetus during Pregnancy

Days of Gestation	Diameter of the horn	Amount of fetal fluid	Length of the fetus	Weight of the fetus	Fetal characteristics
30	2-4 cm	30-60 ml	1 0 cm	0.305 g	Head and limbs bud recognizable
40	3-6 cm	75-100 ml	1.75—2.5 cm	1-1 5 g	—do—
50	5-7 cm	90-200 ml	3.5—5.5 cm	3-6 g	—do—
60	6-9 cm	200-450 ml	6—7 5 cm	8-30 g	Claw buds serotum
70	7-10 cm	350-650 ml	7—10 cm	25-100 g	—do—
80	9-12 cm	500-800 ml	8—13 cm	120-200 g	—do—

Contd.

1	2	3	4	5	6
90	10-13 cm	750-1400 ml	13—17 cm	200-400 g	Hair on lips chin and eyelids
120	12 5-18 cm	2000-3500 ml	22-32 cm	1000-2000 g	Fine hair on eye-brows, claw developed.
150	18-23 cm	4000-5000 ml	30-45 cm	3000-4000 g	Hair on eye-brows and lips, testis in the scrotum, teats developing.
180	——	4000-7500 ml	40-60 cm	5-10 kg	Hair on inside of the ear and, around the horn pits, tip of tail and muzzle.
210	——	8000-10000 ml	55-75 cm	8-10 kg	Hair on meta-tarsal, metacarpal and phalangeal region
240	——	8000-12000 ml	60-85 cm	15-25 kg	Fine short hair all over the body. Incisor teeth not erupted
270	——	12000-20,000 ml	70-100 cm	10-50 kg	Hair coat complete and long, fetus, large incisor teeth erupted.

The growth rates of the fetus and its component organs and tissues vary during different stages in intra-uterine life For example, during early fetal development, the cephalic region grows rapidly and consequently, the fetal head is disproportionately large. Later in gestation, cephalic growth slows down At birth, the head and limbs are relatively more developed than the muscles

CHAPTER EIGHT

Pregnancy Diagnosis

The main purpose of pregnancy diagnosis is to enable the early identification of the non-pregnant females and to reduce the chances of infertility in females This helps the veterinarian to maintain the maximum reproductive efficiency of farm animals By ascertaining early pregnancy, the owner can decide whether the animal is to be kept or culled This will minimize the expenditure incurred on the maintenance of infertile or non-pregnant animals The following are the main procedures adopted for pregnancy diagnosis. These include: Clinical, Chemical, Microscopical, Radiography and Immunological.

Clinical Examination of Pregnancy:
A Majority of cattle owners and veterinarians depend upon this method of examination The main limitation of this examination is that it cannot be used for very early pregnancy diagnosis in large animals and is not suitable for small animals.

The following are the most important symptoms used in diagnosing pregnancy in animals:

1. *General signs*—These are manifested by alteration in temperament, character, aptitude, ceassation of heat and tendency to fatten.
2. *Physical signs*—The volume of the abdomen increases as the stage of gestation advances and the mammary glands start developing at about 30-60 days before the date of parturition in heifers and about 15 days in pluriparous animals.
3. *Milk test*—The milk of the pregnant animals becomes sour, sinks in water and has a high specific gravity.
4. *Urine*—The salt contents of urine are diminished.
5. *Auscultation*—The pregnancy can be diagnosed by hearing the sounds of the fetal heart with the help of a stethoscope
6. *Rectal palpation*—This is a universal method adopted for pregnancy diagnosis in large animals A pregnancy of even 40 days can be diagnosed by experienced veterinarians

The following are the main symptoms observed in pregnant animals during the palpation of genitalia per rectum·

(i) Palpation of amniotic vesicle and fluid feeling;
(ii) feeling of fetal membrane,
(iii) asymmetry of the uterine horns;
(iv) palpation of cotyledons in the gravid horn after 70 days of pregnancy,
(v) feeling of the fremitus in middle uterine artery after 105 days of conception;
(vi) descent of the gravid horn into the abdomen after 3 to 4 months of pregnancy; and
(vii) feeling of the fetus, fetal parts and its movement.

The Detailed Procedure for Rectal Palpation of Genitalia for Pregnancy Diagnosis is as follows:

Prior to the actual rectal examination, the following information either from the breeding records or from the herdsman should be obtained.

1. The breeding history of the animal, including the date of the last calving, the dates and number of previous services.
2. Information on any previous reproductive problem.

Procedure:

(i) The operator should wear proper protective clothing consisting of rubber gumboots, rubber apron/smock and a special long hand glove, short sleeve hand glove Disposable plastic gloves can also be used.
(ii) The finger nails should be closely clipped.
(iii) The animal should be properly restrained.
(iv) The arm of the operator should be well lubricated with non-irritating soap or liquid soap.
(v) The fingers and the hand are inserted into the rectum in the form of a cone.
(vi) The hand should be further advanced gently and the cervix should be located and palpated.
(vii) Try to remove the faecal material (backracking) if necessary.
(viii) The rectal examination should be done with due care, and patience to avoid trauma to the rectum. The mucosal damage may result in bleeding.
(ix) Usually the cervix and uterus is palpated on the brim of the pelvis in older cows and in the pelvic cavity in heifers.
(x) The cervix, uterine body, cornua, and the ovaries can be palpated in the pelvic cavity in the non-pregnant animal and in the abdomen in animals with a pendulous type of uterus.
(xi) The genitalia during early pregnancy will remain mostly in the pelvic cavity.
(xii) During mid-term of pregnancy, the genitalia will have

decended into the abdomen.

(xiii) Sometimes it is necessary to retract the cervix for confirming the pregnancy; in advance pregnancy the cervix as a flat band passes over the brim of the pelvis.

Chemical Tests:

There are many chemical tests but details of some important ones are as under:

1. Sodium hydroxide test—Take 10 per cent sodium hydroxide solution and put some mucus in it and heat it to boiling point. It will turn orange if the animal is pregnant.
2. Barium Chloride test—Take 1 percent barium chloride solution and add urine in equal parts. If there is no precipitation, it indicates pregnancy.
3. Copper sulphate test—Take a saturated solution of copper sulphate and add mucus to it. If mucus turns to a rubber-like paste, it indicates pregnancy.
4. Copper sulphate and milk test—Take 10 ml of 3 percent $CuSO_4$ solution and add 1 ml of milk to be tested. If there is coagulation, it indicates pregnancy.
5. Glucose fructose estimations in mucus—Higher percentage of fructose in mucus (110 ± 20 mg percent) is indicative of pregnancy. In non-pregnant animals, the fructose content of mucus is 35 ± 30 mg percent.

Microscopic Method:

When a mucus smear is examined, the absence of fern like matter and presence of collagen fibre-like reaction, is indicative of pregnancy.

Immunological Method:

The presence of hormones in the pregnant animals can be used as a tool for determining the early pregnancy in animals.

Progesterone in blood and milk:

The concentration of progesterone increases in milk and blood during early (19-25 days) pregnancy. This can be estimated by the radio immunoassay technique.

Radiography Methods:

There are three methods of which the last two are important.

1. *X-Ray*—This is possible only after bone formation. But it can be harmful to the fetus and also to the operator.
2. *Ultrasonic*—This is an instrument used for the diagnosis of pregnancy based on feeling of the fetal heart sound.
3. *Vetscan*: This can be used for diagnosis of pregnancy in all

type of animals In this, the sound waves are projected in the form of images on a screen. A true picture of the embryo or fetus is obtained and, thus, the age of the fetus can be ascertained by measuring the size of the image.

CHAPTER NINE

Mechanism of Parturition and Peripartum Management of Animals

Parturition encompasses the various physiological processes involved in the birth of the young The period of parturtion is, in fact, one of the most crucial stages in the life of any animal, primarily because it is the period of highest death rate in animals It can be a period in which not only the fetus but also the dam may be severely injured, thus affecting the future reproductive and productive potential Hence, the farmer must exercise great care to ensure normal delivery as he invests a large portion of his wealth in animal farming.

Knowledge of the symptoms of approaching parturition will not permit an accurate prediction about the time of parturition in a certain animal but these symptoms are useful indicatives about the approximate time of parturition.

Just prior to parturition, most animals tend to segregate themselves from the others The sow, dog and cat attempt to make a suitable bed. In the cow, the pelvic ligaments, under the influence of relaxin, relax progressively. This relaxation of the ligaments is also noted by the elevation of the tail head The vulva becomes edematous and more flaccid until it is 2-6 times its normal size The udder becomes enlarged and edematous. Just prior to parturition, the udder secretion changes from a honey-like, dry secretion to a yellow, turbid, opaque, cellular secretion called colostrum The cow usually exhibits a tenacious, whitish stringy type of mucus coming from the internal part of the vagina, which starts about the seventh month of pregnancy. This mucus becomes more profuse with the cow approaching calving. During the last few hours, the cow may exhibit anorexia and restlessness. Heifers may show signs of pain, switch their tails and lie down and rise.

Understanding the parturition control should take into consideration the fact that the oviduct has evolved over a million of years from a simple tubular conduit to a way station for the processing of eggs and finally for the development of the fetus Morphologically, the organ has changed to accommodate these functions and in accounting for the diversity, the evaluation has been both parallel and sequential. Many of these functions, particularly as influenced by hormonal hierarchy, have evolved to

adopt to the various differences in reproductive style, and the duration of the process. The hormonal facilitating mechanism is supposed to be acting as a primary regulating mechanism at the cellular level where it influences a chain of biochemical reactions, helping in adaptation to internal fertilization and egg processing. Viviparity and long gestation periods introduced the role of secondary regulation (steroid hormones).and a third level of control, tertiary regulations influencing fetal development and maturation.

The parturition, in general, is an interplay of maternal hormones, fetal hormones and several physical and mechanical factors. According to the usually accepted view of the hormonal control of pregnancy, the contractile activity of the uterus that would otherwise expel the growing conceptus, is inhibited by progesterone Then at term, the inhibitory action of progesterone is withdrawn, allowing uterine contractions to develop to the point where the cervix of the uterus dilates and labour progresses to delivery. In early pregnancy, the source of progestrone is always the corpus luteum (CL) of the ovary, but later in pregnancy, this function of the CL is taken over by the placenta in some mammals. Thus, mammals may be classified into two groups—in one the maintenance of pregnancy is dependent upon CL function throughout the gestation, while in the other, dependence on the CL decreases at some point during pregnancy Viewed in this way, fundamentally different mechanisms controlling parturition could be expected to operate in the two groups, the mechanism of one group being concerned with the control of CL function, the other with placental function. It is now believed that a common mechanism applies to most, if not all, mammals and that species differences express themselves in variations of them rather than in distinctly different compositions.

By a curious quirk of science, a recent view has returned to the earlier suggestion of Hippocrates (460 BC) that the fetus determines its own destiny. This fact has been aptly demonstrated in sheep, goats and cows. Prolonged gestation has been found to occur in sheep ingesting the weed, *Veratrum californicum* which destroys the *pituitary* of the fetal lambs. Prolonged gestation in some cows has been traced to hypoplastic or aplastic pituitaries in the fetuses. Experimental destruction of the fetal lamb's pituitary adrenal axis also results in prolonged gestation indicating that the fetal corticoids secretion is critical for normal calving. This view is strengthened by the observation of increasing secretion of fetal plasma corticoids with approaching parturition in both sheep and cow. In fact, hyperplasia of the fetal adrenal has been found in a group of habitually aborting Angora goats.

Experimental evidence is now available to show that the high levels of fetal corticoids preterm in sheep, is associated with the conversion of progesterone

secretion to estrogen in the placenta, resulting in a decline in circulating progesterone levels. Simultaneously, it is hypothesized that the fetal adrenal corticoids also bring about increased prostaglandin F_2 alpha (PGF) synthesis and release. The rising levels of estradiol and PGF with the concomitant decline in progesterone sets the stage for myometrial contraction.

In the corpus luteum dependent animal, such as the cow and goat, the situation is slightly different. The CL is the major source of progesterone production, at least up to 200 days of pregnancy in the cow, while it is the primary progesterone source throughout pregnancy in the goat. Removal of CL before 200 days of pregnancy in the cow causes abortion with delivery of dead fetus. However, ovariectomy after days 200 of gestation is compatible with the maintenance of pregnancy for up to 70 days, although progesterone secretion falls to 10 per cent of the pre-surgical levels. While placental estrogen synthesis has been demonstrated in the cow placenta no definite evidence is available for its progesterone secretion during late pregnancy. Increased PGF release has also been observed in the cow and goat near term. This increase is also associated with a rise in maternal estrogen levels. It is postulated that in the cow and goat, the increased PGF secretion may effect luteolysis of the CL, besides helping in myometrial contractility during labour.

Associated with the PGF increase, oxytocin levels have also been found to rise sharply during the second stage of parturition. However, it is somewhat difficult to say whether this is entirely associated with the rising sensitization of the uterus by estrogen or PGF or whether it is due to the disappearance of progesterone block. Nonetheless, the consensus is that the uterus becomes critically sensitive to low physiological concentration of oxytocin near term.

The Stages of Parturition:

The act of parturition can be broadly divided into three stages:

The First Stage:

This stage is characterized by the active contractions of both the longitudinal and circular muscle fibres of the uterine wall under the influence of increased PGF and estrogen levels with the concomitant decline in progesterone. Oxytocin is seldom released before the second stage of labour. There is greatly increased activity of uterine musculature in the last 1 to 2 hours before birth. Uterine contractions force the fetal membranes and their fluids against and into the relaxed cervix. During this first stage in the cow, uterine contractions occur about every 10 to 15 minutes and last 15 to 30 seconds. As the stage advances, they increase in frequency, strength and duration until contractions occur about every 3 to 5 minutes. In the bovine and ovine animals no fetal rotation

occurs during this stage. In the mare, the fetus rotates from its dorso-pubic or dorso-lateral position into the dorso-sacral position.

In multiparious animals, the contractions of the uterus occur just nearer to the most cauded fetus, forcing it through the cervix and into the birth canal. Then the same process is repeated for the most cauded fetus in the other horn or the fetus immediately cranial to the one expelled. The longitudinal fibres of the part just emptied contract, but the circular fibres remain relaxed, so that the next fetus may pass through. This shortens the uterus as parturition progresses, so that each fetus is in turn, brought back near the cervix

Fetal Presentation, Position and Posture of the Fetus: The presentation includes (i) the relation of the spinal axis of the fetus to that of the dam—presentations are either longitudinal or transverse; (ii) the portion of the fetus that is approaching or entering the pelvic cavity or birth canal. This portion of the fetus is anterior or posterior in the longitudinal presentation, or dorsal or ventral in the transverse presentation.

The position includes: (i) the relation of the dorsum of the fetus in longitudinal presentation, or the head in transverse presentation, to the quadrants of the maternal pelvis These are the sacrum, the right ilium, the left ilium and the pubis.

The posture signifies the relation of the extremities or the head, back and limbs to the body of the fetus.

Possible variations of presentation and position are given below:

Presentation	*Position*
Anterior, longitudinal	Dorso-sacral
	Right dorso-ilial
Posterior, longitudinal	Left dorso-ilial
	Dorso pubic
Transverse ventral	Right cephalo-ilial
Transverse dorsal	Left Cephalo-ilial

The normal presentation in uniparous animals is the anterior longitudinal presentation, dorso-sacral position with the head resting on the metacarpal bones and knee of the extended forelegs. Birth can take place without assistance if the fetus is in the posterior longitudinal presentation, dorso-sacral position. Other positions result in dystocia. Thirty to forty percent of the fetus in multipara are presented posteriorly and this is considered normal or physiological Since the limbs in multipara are small, short and flexible, their posture is of no importance.

The Second Stage:

This stage is characterized by the entrance of the fetus or fetuses into the dilated birth canal, rupture of the allantoic sac, abdominal contractions, or labour, and the expulsion of the fetus through the vulva In the cow, following the rupture of the allantoic sac, the

amnion is pushed through the cervix and may appear as a transluscent distended membrane. During this second stage of labour; uterine contractions occur in the cow about 4-8 times every 10 minutes and last 80-100 seconds. The point of the greatest and strongest abdominal straining occurs when the fetal head starts through the vulva. During the passage of the fetus through the birth canal, there is a great increase in oxytocin secretion. Almost all animals, as soon as straining commences, lie down. The mare and the sow usually lie out flat with the legs extended whereas the cow, bitch and ewe are likely to lie on their sternum. The time of the second stage of birth in the cow is from 0.5 to 3 to 4 hours. In pluriparous cows, this second stage usually requires 0.5 to 1 hour. Primipara may take longer time, up to 3 hours or more.

The Third Stage:

This is the stage in which expulsion of the fetal membranes and involution of the uterus occurs

Expulsion of the fetal membranes: After the expulsion of the fetus, the uterus contracts strongly for 48 hours and less vigorously and more frequently thereafter. This is necessary to prevent hemorrhage and to aid in the expulsion of fetal membranes. The incidence of placental retention is generally higher in animals in which the young were not allowed to suckle is well known that suckling stimulates the release of oxytocin from the pituitary. In the cow and ewe, the length of time required for the expulsion of the fetal membranes is normally 0.5 to 8 hours. The mare expels its fetal membranes within 0.5 to 3 hours after the birth of the foal. After the expulsion of the fetal membranes in a normal birth, the cervix secretes a rather thick, tenacious mucus that tends to seal the cervix and thus prevents infection gaining entrance to the uterus.

Involution of the uterus, in domestic animals, with the exception of the cow and ewe, has not been extensively studied. The time required for the uterus to involute to its pre-breeding size varies from 12 to 56 days. This period is important because fertility is reduced until the uterus involutes. Uterine involution takes longer in multiparous cows and cows that have had complications at parturition. The cervix usually returns to normal size within 24 to 40 hours postpartum although a retained placenta will delay its closure.

Peripartum Management of Animals:

Prior to parturition the dam is required to be fed properly balanced rations to provide all the necessary nutrients so that at the time of parturition she is neither fat nor thin.. Generally, a light, slightly laxative feed is preferred. For the last 2 to 3 weeks prior to parturition, violence and excessive exercise or work should be avoided. The animals should be segregated from the rest of the herd in clean,

sanitary, comfortable, quiet surroundings (calving box open).

Artificial Interference in Normal Parturition

If parturition is a normal one there is no need for outside aid by an attendant. Such aid is ill-advised. Valuable animals should be observed during the act of parturition so that injury to the new-born is not caused. The after-birth of ruminants should be removed after its expulsion so that the mother will not eat it.

At the time of the birth of the young, especially for the mare, an attendant should be present to remove the amnion that may be wrapped around the muzzle and nose and cause asphyxiation. If respiration in the new-born is delayed, various procedures should be used to stimulate this activity. The mucus should be removed from the nose and mouth. The new-born should be laid on its side, on straw, not on loose chaff or shavings that may be inhaled, with the head and forequarters slightly lower than the hindquarters. Vigorous rubbing of the new-born with a burlap bag, or shaking its head or tickling its nostrils will help bring about respiratıng activity. Occasionally, an oxygen tank with attached short rubber tube has proved useful. The tube is passed through the pharynx, and the mouth and nostrils are closed tightly. Oxygen under moderate pressure distends the lungs. Opening the nostrils and pressure on the chest collapses the lungs, and the process is repeated as often as necessary.

If parturition takes place in a barn or barnyard, particularly if navel infections are prevalant on the farm, disinfection is essential. To be effective, an antiseptic should be applied 1 to 3 times daily for the first 2-3 days following birth. Tincture of iodine, 1:1000 solution of alcoholic sublimate, 5 percent tannic and 5 percent salicylic acid in 70 percent alcohol, or any similar antiseptic may be applied by carefully soaking the navel stump and squeezing it with cotton soaked in antiseptic. Clean, sanitary, well bedded stalls for the calf and the parturient cow help greatly in preventing navel infections.

The new-born should nurse and thus get the colostrum within 1 to 2 hours after birth. Under highly insanitary, infected environmental conditions, it is desirable to give colostrum to the new-born promptly, within 15 to 30 minutes after birth If necessary, this may be given by a stomach tube. Colostrum is an important source of immunoglobulins which are absorbed from the intestines during the first 24 to 36 hours of life. Additionally, it has laxative properties and is a richer source of proteins (5 times), fat and mineral (2 times) and iron (10 times) as compared to ordinary milk.

The new-born animal should be fed every 1 to 3 hours for the first day or so and then every 4 to 12 hours thereafter. The amount of milk fed daily to the young should be about 10 percent of the body weight. The orphan young should be encouraged to eat solid food as early

as possible so that the amount and frequency of milk feeding can be reduced. Care and nursing are particularly important in the proper rearing if the orphan young is on an artificial diet. Diarrhoea, if developed, can be controlled by reducing the feed intake and by the administration of streptomycin, neomycin, tetracyclin or other antibiotics. Orally a dose of vitamin A and D is also necessary

Care of the Post-Partum Dam:

Following parturition, the dam should be confined to a quite place to avoid undue excitment Rest and quiet following parturition are imperative. The roughages fed to large animals should be of good quality. The grain ration should be rather laxative and light. The amount of grain should be increased gradually during the first 3 weeks after parturition. In the dairy cow it may have to be increased more rapidly, to prevent acetonemia or ketosia The cow should be watched carefully for several days after calving for symptoms of milk fever. Excess oedema of the udder should be controlled by massage and frequent milking

In domestic animals, retention of the fetal membranes occasionally occurs. The membranes are considered as being retained if they are not expelled within 8 to 12 hours in the case of the cow, 3 to 6 hours in the mare and 8-12 hours in the ewe. Veterinarians prefer to treat retained placenta in the cow within 1-3 days after parturition.

If genital discharge persists beyond 14-20 days postpartum or if it is abnormal or purulent in nature, the genitals of the animal should be examined and treated for uterine infections. It is desirable to examine the genital tract about 30 days postpartum in valuable cows, even if the calving and postpartum period is apparently normal. Early treatment of uterine infection is imperative if the animal is to conceive again promptly.

Agalactia or a lack of milk after parturition may be due to the failure of milk let-down or failure of milk production. This condition is noticed occasionally in heifers with greatly congested, oedematous, painful udders. Injection of pituitorin or oxytocin intravenous/intra muscularly can cause rapid and complete milk let-down

PART II

ARTIFICIAL INSEMINATION

CHAPTER TEN

Sexual Behaviour and Libido in Males

The sexual behaviour of animals plays an important role in reproduction. Various patterns of courtship, display, motor activities and postures are directed to bring the male and female gametes together and ensure fertilization.

The components of copulatory patterns are sexual arousal, courtship (sexual display), erection, penis protrusion, mounting, intromission, ejaculation, dismounting and retraction. The duration of courtship and copulation varies with species. Courtship in the male consists of tactile stimulation of the female by muzzling and licking the perineal region. In the presence of a female in heat, the male attempts several mounts. Necessary fluids are excreted by bulls during mounts (dribbling). The male, after mounting the female, fixes his forelegs around her, grasps her firmly and performs rhythmic pelvic thrusts During intromission, the particular abdominal muscles of the males contract and his pelvic region comes in direct contact with the external genitalia of the female. The duration of intromission varies widely between species.

The semen is ejaculated near the OS-cervix. After ejaculation, the male dismounts and the penis is soon retracted into the prepuce. Most of the males show no sexual activity immediately following copulation. Frequency of copulation varies with the species.

A set of hormones called 'Pheramons' are scouted from the animal. They are odoriferous compounds which act as sex attractants and regulate sexual behaviour.

There is an endocrine, neural and genetic control of the sexual behaviour. The gonadal steroid balance serves as the origin of sexual motivation. The hormones, transmitted through the blood, activate the central nervous system. The hormonal signal is transformed into sexual motivation or sex drive. The sensory information allows the initial searching for sexual partners and releases the appropriate motor reactions.

The partners intensity of sexual behaviour is affected by genetic, physiological and environmental factors as well as by previous experience. There are breed and individual differences in the amount of sexual stimulation and these can be attributed to the genetic influence The effect of external stimulation on sex-

ual behaviour is more pronounced in the male than in the female

Sexual activity of the male increases when new females in the herd become receptive. That is why, changing of the teaser cow is a very effective way of increasing the sexual behaviour of a sluggish male. The presence of other males, while teasing a female improves the sexual libido of the male. Dominant males perform better. Seasonal variations, when any, in the sexual behaviour, are mostly due to seasonality of pituitary function, controlling the secretion of gonadal hormones. The efficiency of copulation in males is improved by experience. The presence of males has an effect on the duration of estrus and time of ovulation in females. Precoital stimulation affects both composition of the ejaculate and male hormone secretion.

Libido means sex drive. Under controlled setting libido has been measured in terms of 'reaction time' which is defined as the time taken by a male from his first contact with a female to the moment of ejaculation. Sexual intensity has also been measured as the total number of mounts, both with and without ejaculation, or the latency of ejaculation.

The most meaningful measurement of the sex drive are (i) the number of ejaculations during a constant period of time or until no more ejaculations can be collected in a reasonable period of time with all available stimuli (depletion or exhaustion test) and (ii) the latency of ejaculation (reaction time).

Male sexual behaviour is markedly reduced in intensity during periods of pysiological stress caused by disease, low plane of nutrition, or climate extremes. Pathological conditions reduce sexual expression. Similarly deficiencies of vitamins and minerals reduce libido.

CHAPTER ELEVEN

Artificial Insemination– Advantages and Limitations

Artificial insemination (AI) is the most important single technique ever devised by man for the genetic improvement of domestic animals. This technique has made it possible to breed a few highly selected males to thousands of females every year. The AI technique makes available sires of high genetic inheritance for, milk and butter fat, increasing the usefulness of such superior sires to an extraordinary degree.

Through natural breeding, only a small number of animals could get the advantage of good bulls for crossbreeding programmes in the country. The AI technique has found wide applicability. It is commonly seen that a bull can be bred to 50-60 cows by natural services per year, while through AI, there is a record of a single bull having been bred to 50,000 cows. Commonly, a single bull can easily be bred to around 1000 cows annually through AI and with the advent of the frozen semen technique,it has become possible to breed 10,000 cows.

Legend has it that the AI technique had its origin in 1322 at which time an Arab tribal chief used the artificial method to impregnate a prized mare with semen collected from a stallion belonging to an enemy tribe. In 1870, L. Spallanzani proved the fertilizing ability in the spermatozoa This was the first scientific research in AI in domestic animals. A bitch which was artificially inseminated, gave birth to three pups. And all the pups resembled their mother and the dog whose semen had been used. In 1782, P. Rossi and Prof. Branchi successfully repeated the experiment of Spallanzani.

The AI technique was applied some years later to women by Hunter (1799) and to horses by a veterinary surgeon, Repiquent, in 1835. Heape in 1897, wrote that in the light of the work carried out on bitches between 1884 and 1886, it appeared that AI was easy and that conception was as readily induced in that way as by the normal method and that one ejaculation served several females. It was claimed that this method could be used to cross dog breeds whose natural mating is impossible. Artificial insemination was first used to horse breeding in Europe in 1890 when Repiquent advised its use as a means of over coming-sterility.

The best known Russian investigator and a leading pioneer in artificial insemination at the turn of century (1899) was E.I. Ivanoff. Under his direction AI was practised by many horse breeding stations with limited success. He was the first to undertake successful AI in cattle

and sheep His results created great interest, thus making the USSR a pioneer in artificial insemination work in domestic animals. By 1938, the number of animals thus inseminated in Russia rose to 1.2 million cattle, 15 million sheep besides 120000 mares During the same period, Edward Sorensen in Denmark organized the first artificial breeding association. J.A. Henderson (1931) introduced AI in Minnesota (USA) and Howard Clapp in 1938 proved that a large number of cows could be bred through AI with good success. Subsequently, in Sweden (1943), and Norway (1982), AI organisations were established. The technique has been used most widely for breeding cattle More than 60 percent of the dairy cows in theUSA and nearly all cattle in Denmark (98 percent) and Japan (92 percent) are inseminated through the AI technique.

In India AI was first attempted in 1939 by Dr. Sampath Kumaran at Mysore. The Indian Veterinary Research Institute, Izatnagar started experimental work under the direction of Dr. P Bhattacharya in 1942. By 1948-1954 AI work had spread to most of the states in the country. It received a great boost from 1951-66 by the launching of a master project of the key village scheme, in which AI was accepted as a major activity in livestock improvement programmes. The programme was further extended to wider areas under the Intensive Cattle Development Project (I.C.D.P.) These projects were started in 1966-67. Each project covered a population of about 100,000 breedable cows and buffaloes.Artificial Insemination has been accepted as a major activity for bringing about rapid genetic improvement In recent years, AI has been organized on a cooperative basis by a large number of milk producers unions in many parts of the country.

Advantages of Artificial Insemination:
It has amply been proved through livestock development plans for cattle and buffaloes that the method of artificial breeding has distinct advantages over natural breeding. The technique has tremendous scope in increasing productivity of Indian cattle and buffaloes through improvement in their genetic constitution, by large scale introduction of semen from outstanding bulls. The major advantages of AI are as follows:

1. *Quick genetic improvement:*
Widespread use of outstanding bulls offers a great opportunity for genetic improvement in dairy animals. Through the use of frozen semen a bull can serve as many as 500,000 cows in his lifetime. And even after the death of that bull, his semen may be used. Availability of semen gives an opportunity to the breeder to select superior bulls for animal improvement This can also help in early selection of superior bulls through progeny testing programmes, as one animal can be tested on many females.

2. *Control of veneral diseases:*
Spread of diseases can be effectively con-

trolled through AI since there is no direct contact between male and female Further, each bull is examined before collection of semen. This eliminates the chance of use of an infacted bull.

3. *Semen evaluation*:
Through this technique the semen to be used for breeding can be evaluated and its quality determined. Quality semen producing animals can, thus, be easily identified

4. *Crossbreeding*
This method has enabled dairy farmers and animal husbandry workers to obtain crosses between different breeds or species. What used to be impossible in terms of mating breeds of unequal size, has become practically possible.

5. *Maintenance of records:*
Accurate breeding records can be maintained because of controlled breeding.

6. *Economic rearing of animals:*
Economics is a major factor in livestock production. The cost of using outstanding sire through AI is always less than through natural mating. Maintenance of a large number of mediocre bulls is uneconomical and unprofitable.

Limitations of AI Technique :
This technique, though having tremendous advantages, suffers from certain limitations if the technique is not implemented properly.

1. *Limits to detect animal in heat:*
The technique of AI brings in its wake the human factor of picking up animals in estrus and judging the proper time for insemination. This in natural mating is effectively done by the bull.

2 *Requires trained technicians*:
AI programme requries a certain amount of skill This means training is required to be imparted to AI technicians. Though breeding through AI is not complicated, it has been found that a small percentage of people who attempt to learn it never succeed in doing so.

3 *Selection of poor sires accentuates the damage:*
When a bull used in AI is of poor quality undesired types of offspring are born. For this reason, untried or untested bulls should not be used. This precautionary measures virtually eliminates the possibility of spread of genetically inferior dairy animals.

4. *Large scale spread of disease*:
While certain diseases are eliminated through AI it also leads to large scale damage if the bulls selected for breeding are afflicted with diseases.

5. *Requires special equipment*:
With the advent of the frozen semen technique, costly equipment, such as liquid nitrogen containers, good quality straws, etc. need to be used for the success of the programme. Nonetheless, the advantages always outweigh the limitations.

CHAPTER TWELVE

Semen Collection and Evaluation

Proper harvesting and scrutiny of male germ plasm is the first essential prerequisite of the artificial insemination programme. In the absence of a near natural semen collection method, the whole programme will collapse owing to poor quality and quantity of the spermatozoa so collected. A sound and hygienic semen collection technique followed by proper evaluation is a must for maximum utilization of semen with desired results in the artificial breeding programme.

Collection of Semen

Several methods of semen collection from a bull have been tried from the beginning of this century In the early methods of collection the bull was allowed to copulate naturally and the semen was scooped out from the vagina or a sponge was placed in the vagina of the cow and semen squeezed out afterwards. The semen could be collected by these crude methods but was highly contaminated with the secretions of the female tract and microorganism. Some improvised techniques like a rubber bag placed in the vagina did not result in enough ease in the collection. With the invention of the artificial vagina, the most practical and satisfactory means of collecting semen was achieved.

There are at least 3 methods of semen collection generally used for collecting semen from a bull. These methods are (i) artificial vagina; (ii) massage of seminal vesicle and (iii) using electric ejaculator. There is no doubt about the superiority of the artificial vagina method but obtaining semen by massaging seminal vesicles and ampullae or using an electric ejaculator have their merits specially in the case of bulls with a lack of libido or those that are unable to mount because of any other reason.

Artificial Vagina Technique :

It is more than clear that without this system, artificial insemination would not have developed to its present dimensions. This has made the semen collection act near natural for the bull. The artificial vagina (Fig. 12.1) consists of a hard rubber cylinder 12" to 16" long. On the body of the cylinder is fitted a double valve system for warm water and pumping air. A soft latex lining is fitted inside so that a jacket is formed between the cylinder and lining wall to hold warm

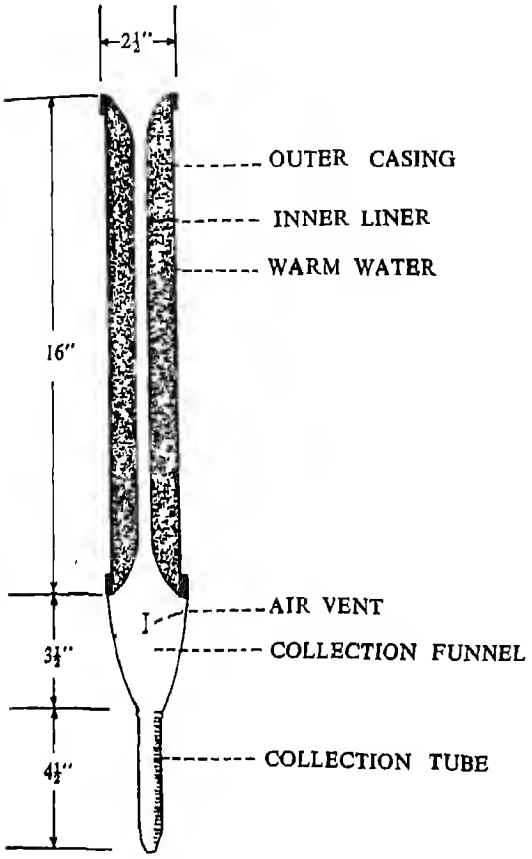

FIG. 12.1 A MODEL OF ARTIFICIAL VAGINA

water and for proper pressure. A latex cone is fitted to one end and a graduated tube to receive semen on other end.

An artificial vagina must be clean, sterilized and dry before it is used for the collection and not more than one collection should be taken to avoid any possibility of contamination. The system is assembled by inserting soft inner lining into the cylinder and the ends are rolled inward and mounted on the ends of the hard cylinder Care is taken to give more folds on the side which will receive the thrust at the time of collection. The wide end of the receiving rubber cone is fitted to other end. A clean sterilized graduated vial is fixed to it. The jacket formed by the cylinder and lining is filled with warm water (42-45°C) and proper pressure is maintained by pumping air. The rubber one and semen tube is covered by an insulating bag to protect the ejaculate from external adversities like high/low temperature, sunlight etc.

Semen collection by an artificial vagina requires a mount animal For this purpose, the bulls are usually trained to mount other bulls. A buffalo bull is generally preferred as a teaser as it is not disturbed by the other bulls mounting on it. It does not feel restless and there is no frequent micturition or defecation as is common with a cow bull or a cow not in heat A cow in heat should not be used as a mount animal otherwise the bull will refuse to mount if a cow in heat is not provided

During collection the artificial vagina should be held at an angle to the ground so that its long axis is parallel to the line of the penis The collector should firmly grasp the sheethed portion of the penis to guide the penis into the artificial vagina. Necessary stimulation to the bull before taking a collection is a must for obtaining better quality and quantity of semen For this individual characteristics of the bull should be known to the collector

Mechanical Massage Technique

The only advantage of this method is that the semen of some valuable bull which are unable to mount because of some physical problems or poor libido, can be obtained. This is achieved by inserting the hand into the rectum and pressing and massaging the seminal vesicle and ampullae. The bull ejaculates after about 2 to 3 minutes' massage. The semen obtained by this method is usually of poor quality and more diluted as a greater amount of seminal vesicle fluid is secreted due to the massage. There are also, chances of contamination with urine.

Electric Ejaculation Technique :

A mild alternating current applied to the sacral and pelvic nerve by electrodes placed in the rectum, induces ejaculation in a bull. This method is commonly used for small animals like the sheep and goat and is restricted to bulls with poor libido or to those which are incapable of mounting due to physical disability or old age. The semen obtained is thin because of enhanced secretion from accessory glands. However, the quality of semen is superior as compared to physical manipulation. If the method is applied properly the animal does not experience any harmful effect except a temporary tetanic contractions of all body muscles.

Evaluation of Semen:

The success of artificial insemination/ depends upon the availability of good quality spermatozoa in optimum strength. Although the acid test for spermatozoan is its fertilizing ability, certain simple tests can depict the probable potential of the semen. This is an added advantage in artificial breeding programmes as the use of semen showing poor quality can be eliminated. Proper quality evaluation is also important for optimum utilization of semen with the best results.

Semen evaluation can be broadly divided into two parts: 1. The routine examination of the semen should be carried out immediately after the semen is collected. This should be quick as delay in processing will have a detrimental effect on the ejaculate. In such an examination the overall appearance of the sample is to be seen. This will include observing ejaculate volume, mass activity or collective movement of spermatozoa, motility and spermatozoa concentration in the semen. These observations provide a gross reproductive performance and lead in taking decisions regarding the number of doses to be made and extension rate to be used for the ejaculate. 2. Other evaluation procedures are time consuming and cannot be used as routine. These methods involve morphological, chemical and biochemical studies. These tasks are employed initially to select a bull for the programme, and periodically, to see whether the quality of the spermatozoa is maintained. This type of examination involves determining morphology of normal sperms, staining for

live and dead sperms by differential stains, study of metabolic performance, level/leakage of various enzymes and finding out the resistance of spermatozoa to adverse conditions.

The following observations are must for the examination of semen:

General appearance: Immediately after the collection of semen it should be checked for any foreign material like loose hair, dung, dirt or pus, etc. Bull semen is usually milky white, varying to creamy or lemon colour. Ejaculates showing any deviation from the normal colour for a particular bull should be discarded

Volume: A cattle bull on an average gives 4-7 ml semen for each ejaculation. There should not be a great variation in the quantity of a particular bull. In case the volume is unusually large, it may be because of mixing with urine which could be checked by smell, or leakage in the A.V Too small a volume compared to the normal volume ejaculated by a bull may be because of less sexual stimulation provided by the collector or sometimes due to sickness or physical injury to the bull. Recording the volume of semen is necessary from the point of view of deciding total extension.

Mass activity. Collective movement of the spermatozoa in the semen observed under the low power of a microscope is termed as mass activity In a drop of normal semen under the microscope, a wave motion depending upon the number of sperms is seen The intensity of the movement of the wave depicts the quality of semen. Multi-directional, fast moving waves giving an impression of clouds represent the excellent quality of semen in terms of motility and sperm concentration. Semen with slower intensity of waves is relatively poor in quality. Semen showing sluggish waves, no waves with individual sperms moving or no movement indicate very poor quality and should be discarded. A numerical grading is given from +1 to +5 depending upon the intensity of waves. Semen samples showing gradation below +3 are generally not used for the artificial breeding programme. For proper observation of mass activity or sperm activity it is essential to use a microscopic stage heated to 37°C.

Sperm concentration: It is very essential to know the number of spermatozoa present in the ejaculate for the maximum use of the semen. Dilution rate can only be decided after knowing the number of live sperms in the semen Enumeration of spermatozoa, in the routine, is carried out by a photoelectric colorimeter. The number of sperm cells in the semen are directly proportionate to the amount of light they will absorb at a particular wave length Semen is diluted in a buffer to a ratio of about 1·50 A cuvette or a small glass tube with the diluted semen is placed in the photoelectric colorimeter and the light absorbed as compared to the buffer without semen is recorded. This method requires standardization of

the colorimeter to be used, with a haemocytometer, a device used for counting blood cells

A haemocytometer can also be used for the routine enumeration of sperm cells but the technique is time consuming and the results cannot be effectively used as these are not available immediately. The cell packed volume method can also be used, but is not practical in routine work.

Motility: Assessment of individual motility of the spermatozoa and percentage motile spermatozoa are observed under the low power of a microscope. A small drop of diluted semen is placed on a clean slide and is covered with a cover slip. The slide is placed on the warm stage (37°C) of the microscope for examination. The per-centage of progressively forward moving spermatozoa is estimated by seeing a number of fields Semen with motility percentage below 50 percent or a high percentage of abnormally moving sperms (above 15 percent) should not be used for the AI programme.

The dilution rate of semen is decided on the basis of sperm concentration and motility percentage to give the minimum number of live sperms per dose for insemination.

Tests not routinely used consist of differential staining of spermatozoa for live and dead count Certain stains can penetrate the plasma membrane of dead sperms while live sperms are not affected. The stained slide can also be used for observing the number and types of abnormalities present in the semen. Bulls with more than 15 percent primary abnormalities are not used for breeding. Periodical observation of acrosomal integrity can also be carried out by using stains for acrosomal cap. Certain enzyme estimations are also used as an index of sperm integrity or that of its different sections.

Stress tests like giving temperature shock to the semen by placing it in ice or giving excessive dilutions, could be used initially and occasionally to test the preservability of the semen.

Extension procedure for semen should ensure that the spermatozoa with their fertility function are available in optimum number and in convenient volume for insemination. A proper extension technique is important for keeping the spermatozoa viable and functional.

Semen should be immediately diluted after collection and primary evaluation. The extender is directly added in the semen tube after taking the necessary amount of semen for enumeration of spermatozoa and other tests. A small dilution is made on the basis of motility and sperm concentration The initially diluted semen is poured into the diluent directly. The volume is made keeping around 20 million live sperms per dose. It is important that the semen and diluent should be at the same temperature to avoid temperature shock to the sperms. Pouring should be done directly into the

extender and not by the side of the glass vessel containing it for the same reason

The extension procedure for freezing semen is modified as the important cryoprotectant factor is to be incorporated in the extender before freezing The extender is divided into two equal parts. To one part, semen is diluted as usual and to another part, glycerol is mixed Both the fractions are cooled to 5°C slowly. The final dilution is done at 5°C by adding glycerolated extender to extended semen.

In case of certain exothermine buffers like tris, glycerol can be mixed initially with the whole extender and semen can be extended at room temperature without any deleterious effect on the spermatozoa.

CHAPTER THIRTEEN

Semen Extenders

The volume of semen collected from a bull by the use of the artificial vagina averages about 5 ml. Each ml of semen, on an average, contains about 1,000 million spermatozoa. It has been established that 10-20 million live spermatozoa in a volume of about one ml will yield a satisfactory conception rate when inseminated artificially It is apparent, therefore, that the average ejaculate from a normal bull can be used for the insemination of a large number of cows if the volume is increased by dilution.

The ideal medium should not only extend the volume of the ejaculate but also be favourable for the survival of the spermatozoa. A large number of media have been developed incorporating requirements for nutrition, protection against microbial contaminants and also temperature shock, as well as the proper pH, isotonicity and buffering capacity. Above all, the osmotic pressure and effects of various electrolytes and non-electrolytes on semen must be considered. Besides extenders should be easy to prepare, economical and stable

The most significant improvements in semen extenders over the past three decades have been the discovery of the protecting influence of egg yolk, the use of milk and milk products and the incorporation of antibiotics in the diluent to control microbial contaminants and to minimize the possibility of the spread of infection through artificial breeding.

Preparation:

Reagents used in the preparation of semen extenders should be chemically pure and should be prepared by using sterile, pyrogen free, double distilled water. Every piece of equipment should be properly cleaned, rinsed with double distilled water, dried and sterilized. Physiological saline or sugar solutions are used for increasing volume and for immediate use of the semen. A preserving extender is developed only after knowing the merits of egg yolk and the reduction in metabolic activity of spermatozoa at 5°C, thus increasing their life.

Egg yolk extenders· For the preparation of these extenders, freshly laid eggs from healthy flocks are selected. Any egg containing blood or meat spots is discarded. The egg shell should be wiped clean with alcohol before breaking Care should be taken to separate the yolk from the white and also to remove the membrane which

covers the yolk. This is accomplished by using sterile blotting paper and forceps

Egg yolk Citrate: A 2.9 percent solution of sodium citrate is prepared, in double distilled water and sterilized in an autoclave. When cool, 25 percent egg yolks, freshly prepared are added to it. Antibiotics are usually added to give a concentration of 1,000 IU penicillin and 500 to 1,000 micrograms of streptomycin per ml of extender. Egg yolk has been used with varying amounts (10-50 percent) with comparable results Some artificial breeding centres have used 3.2 percent and 3.6 percent solutions of sodium citrate rather than 2.9 percent with little or no apparent difference These egg yolk buffers are relatively harmless to cell suspension and provide excellent buffering capacity over a suitable range of pH. Tris salt (N-tris hydroxymethyl amino methane) has been found ideal as a buffer for preservation of spermatozoa. In combination with egg yolk, it gives equal or better results, in terms of preservation and fertility as compared with egg yolk. The following composition can be used for cattle semen dilution

Tris	3.028 g
Citric acid monohydrate	1.675 g
Fructose	1.25 g
Distilled water	100 ml
Egg yolk	25 ml

Antibiotics are added at the usual rate.

Milk Extenders:

In recent years, milk extenders have become very popular for routine artificial insemination in western countries These extenders are economical, easily prepared, offer excellent protection to spermatozoa, and the fertilizing capacity of semen so extended has been satisfactory The greatest drawback to their use is that they are very opaque and the presence of fat globules make microscopic examination difficult for the inexperienced worker in the absence of a good phase contrast microscope

Whole milk extender. Cow milk with a butter fat percentage of 3 5 percent forms a good extender. The milk is heated indirectly in a water bath The temperature of the milk is brought up to 92°C and it is kept at that temperature for 10 minutes The milk is then cooled, after which it is filtered to remove the coagulated protein formed Penicillin (1,000 IU) and streptomycin (500 micro gram) are then added to each cubic centimetre extender

Skim milk extender This extender is prepared in the same way as the whole milk extender Microscopic examination is easier because of the absence of fat globules. However, some workers indicate better protection and conception rate with whole milk diluents.

Powder milk extenders · A solution of 10 per cent (weight/volume) skimmed milk powder in distilled water is prepared in the same way. Milk is to be kept at 90°C

for 10 minutes Roller dried milk does not need this treatment

Detoxified milk diluent In place of heating, the toxic element of milk (lactanin) can be denatured by adding cystine hydrochloride 1 mg/ml of milk.

Milk whey extender: A diluent for buffalo semen has been evolved at N.D.R.I. Karnal. The diluent is available in a packet formula which contains 10g roller dried milk and 0.6g of citric acid monohydrate. When the contents are suspended in 100 ml of distilled water, the milk gets curdled as the citric acid lowers the pH. The contents are filtered and the filterate whey is used as a diluent after adjusting pH to 6.8 and adding antibiotics at usual rates.

Extenders for Semen Freezing:

All the above diluents are used for freezing of semen with addition of glycerol which is a remarkable cryoprotectible agent in semen preservation The level of glycerol recommended varies with different workers but it ranges from 3-10 percent The optimum concentration of glycerol for any of the above diluents is 6 to 8 percent.

Spermatozoan survival is better if the glycerol is mixed with the semen at 5°C as spermatozoa get damaged if glycerol is added to semen at a higher temperature. Therefore, for freezing semen, the total extender is divided into two equal parts To one part, semen is diluted, and to the other, double the amount of glycerol is added to make the optimum concentration of glycerol at final mixing Both are cooled to 5°C and the glycerolated diluent is added to the diluted semen in 3 to 4 fractions, at intervals of 10 minutes. It has been shown now that at 5°C glycerolated diluents can be added with any deleterious effect on spermatozoa. With egg yolk and tris buffer, glycerolisation can be carried out at room temperature without significant damage to sperm.

Sugar, besides giving energy to the spermatozoa, has been found very useful in improving post-thaw motility of frozen semen. One to two percent fructose provides substitutes for electrolytes in maintaining the osmotic balance of the extender.

A disaccharide like lactose is used specially for pellet freezing, as the sugar replaces some of the buffering salts of the extender and is, thus, beneficial for quick freezing.

Extension of Semen

The objective of extending semen to a prescribed amount is that the appropriate volume of semen inseminated will contain sufficient sperms to give higher fertility without wastage of sperms. As low as 5 million sperms per dose has been found enough for conception. A sperm number of 10 to 20 million should be kept per dose to get better results, specially in field conditions. As about 50 percent of spermatozoa are damaged in freezing, double the number of sperms should be kept per dose. Dilution will also depend upon the freezing method

used. Depending upon the technique, which may be French mini or medium straw, land shut mini tube or ampoule, sperm concentration per ml is to be decided. In pellet freezing, final dilution is made at the time of thawing and a dilution of 1:2 to 1:4 is carried out before freezing.

A ready reckoner is generally used for deciding dilution rate depending upon sperm concentration and initial motility for different types of containers used.

CHAPTER FOURTEEN

Principles of Semen Preservation

Preservation of semen is an essential component in any good artificial insemination programme. In order to propagate production of high quality offspring, preservation of genetically and physiologically worthy spermatozoa for indefinite periods of time is imperative.

Theoretically, semen preservation could be brought about either (i) by providing an isotonic medium containing all essential metabolic ingredients, such as nutrients, enzymes, coenzymes, tonic substances and vitamins, or (ii) by bringing about inhibition of all but the minimum essential cellular activities. Of the two extreme methods, the first is very tedious requires a complete knowledge of cellular functions and the means for fulfilling these needs, neither of which are available at present. Because of the relative ease with which metabolic processes can be supressed by lowering of temperature, and the general reversibility of the process, the second method is universally accepted.

Preservation of Semen:

Temperatures below and above freezing point: It should be borne in mind that physiological temperature limits for bull spermatozoa, that is, the temperature range at which spermatozoan life can exist, varies from at least -196°C (the temperature of liquid nitrogen) to 50°C. Within these limits, other things being equal, the higher the temperature, the faster the metabolism, and above 7°C, the faster the motility of cells, the shorter their lives.

Temperatures at or above that of the body: Where refrigeration facilities are non-existent, semen could be preserved at room temperature though for a maximum period of only 2 to 3 days, depending upon the ambient temperature. It is also very essential that the semen be extended in a diluent containing adequate energy yielding substrates like glucose and fructose The reason is that sperm cells have a limited life span at warm temperatures, besides having a limited fuel supply and limited capacity to absorb or detoxify end-products. It may be hypothesized that some of the essential constituents required for metabolism are not being resynthesized and the cell membrane becomes impermeable to these constituents. Some of the diluents which have been used for extending semen at ambient temperatures

include the Illinois Variable Temperature (IVT) extenders, the Cornell University Extenders (CUE), Caprogen and Coconut Milk Extender (CME).

Temperatures below that of the body : As the temperature is lowered below that of the body, the rate of metabolism and the motility of cells decreases, though not necessarily at the same rate. At 7°C all visual motility ceases, yet metabolic activity continues at a rate which limits the time period over which the fertilizing capacity can be maintained. As the temperature decreases from body temperature to 0°C, the solubility of the gases O_2 and CO_2 increases 2 to 3 fold. With air as the gas overlying the spermatozoa, this shift in solubility of gases at lower temperatures permits a relatively greater proportion of the total metabolic activity due to oxidative metabolism.

This results in a relatively greater oxygen supply to the spermatozoa. Additionally, the solubility of certain solutes decreases and the physical form of the colloids in the semen change, causing an increase in the viscocity of cytoplasmic contents, resulting in greater physical resistance to motility. The decline in metabolic activity, increase in solubility of oxygen and fall in motility, all help in increasing sperm longevity at low temperature.

Most semen used for inseminations prior to the availability of frozen semen technology was maintained at refrigerated temperatures (4-5°C). The first extenders in use were the egg yolk phosphate based media (EYP), egg yolk citrate (EYC), and various others such as citric acid whey (CAW), tris-buffer, glucose, soda-bicarbonate buffer, glucose and sodium citrate, heated skimmed milk extenders

Unfortunately, it is not possible to preserve semen by bringing down the temperature suddenly, as the effects prove harmful. If an undiluted semen sample in a test-tube, previously held at body temperature, is suddenly plunged in an ice-bath and held there for a few minutes, it would be seen that the percentage of cells which regain motility is greatly reduced. This effect is termed as cold shock which causes leakage of intracellular enzymes, potassium, lipoprotein, ATP and other materials from the cells. The precise mechanisms of damage are unknown but presumably changes occur at unequal rates on the surface and internal portions of spermatozoa during cooling, and both physical and chemical damage results. In order to prevent cold shock, a gradual cooling of semen is essential with the time taken for bringing down the temperature to around 5°C being at least 1 to 4 hours. Addition of lecithin, proteins, lipoproteins and similar complexes found in egg yolk or milk also help in preventing cold shock.

Temperatures below freezing point: For long-term preservation of semen, freezing is essential. During freezing and thawing of semen, the motality of spermatozoa could be extensive. The damage is caused by:

1. Internal ice crystal formation that affects the structure of spermatozoa
2. The solute concentration as pure water is withdrawn from suspension media both inside and outside the cell.
3. The interaction of these two physical factors.

The major physical and chemical consequences of freezing are the removal of pure water from solution to form ice and the resultant increased concentrations of solutes in the residual liquid. For reducing ice crystal formation, the freezing rate should be slow, about 0.8 to 3°C/minute from +5 to -15°C and 3 to 5°C/minute from -15 to -79°C. These rates result in the formation of a relatively small number of large extracellular ice crystals.

Glycerol Function

For cryoprotection against ice-crystal formation, glycerol addition has been found to be very useful, primarily through its water binding capacity and decreasing the freezing point of solutions, thereby allowing less ice to be formed at any given temperature. Consequently, the solute concentration in the residual liquid is correspondingly reduced. Other cryoprotective agents like ethylene glycol and propylene glycol have been tried but their effectiveness is much less as compared to glycerol.

Qualities of Semen Extenders for Freezing:

For maximum utilization of semen collected from a proven bull for a large number of inseminations, its dilution in a suitable semen extender is very essential. Mixing spermatozoa with extenders allows the addition of many ingredients that sustain and protect the spermatozoa, thereby preserving fertility until they are used for insemination. Thus, the two major functions of semen extenders are to preserve the fertility of sperm cells and to increase the total volume so that the proper dose of cells for insemination can be conveniently packaged and used. A single ejaculate can, therefore, be used to inseminate several thousand cows.

The basic components of any extender for preserving bull spermatozoa are:

1. Water which is a solvent for seminal and extender components
2. Dissolved ionic and non-ionic substances to maintain osmolality of the buffer and to buffer the pH of the medium.
3. Organic materials with the capacity to prevent cold shock (generally egg yolk or milk).
4. Cryoprotective agents such as glycerol.
5. Simple sugars for an energy source.
6. Antibiotics to control microbial growth

Buffers and Non Ionic Substances:
A variety of buffers such as tris, egg yolk citrate, sodium citrate dihydrate and monosodium glutamate have been used A satisfactory extender for one animal or one set of conditions may not be acceptable to others. Thus each laboratory must determine which extender, under its conditions, will provide maximum reproductive efficiency. Osmolality—the number of particles suspended as solutes in a solution—influences not only, the osmotic pressure of a solution but the point at which the solvent freezes. Thus, the lowered freezing point of a solution reflects its osmolal concentration of particles. An osmolal concentration or 1,000 milliosmoles of a solute in water depresses its freezing point by 1 86°C. The freezing point depression of bull semen is approximately —0.55°C, equivalent to a concentration of nearly 300 milliosmoles.

Spermatozoa need protection from antotoxication due to acid products of metabolism, particularly when they are stored without refrigeration. Bull sperm motility and fertility are well preserved in egg yolk and milk extenders near neutral pH, although reduction of the pH to 6 5 has been found to be beneficial.

Organic materials· Egg yolk is a valuable constituent in preserving fertility and has been extensively used in semen extenders. Extenders containing skimmed milk and glycerol have also been developed. Egg yolk and milk prevent cold shock due to their protective constituents—lipoprotein, phospholipid and lacithin in the case of the former, and casein in the latter. The nature of cold shock preservation by these agents is not understood.

Cryoprotective agents: Glycerol is the most widely used cryoprotective agent for bull spermatozoa.

Sugars: The monosaccharides like glucose and fructose are added as energy source for spermatozoa. Additionally, they are also good substitutes for maintaining the osmotic balance of the semen extender

Antibiotics to Control Microbial Growth:
Egg yolk and other extending media for bull spermatozoa provide a good environment for the growth of microorganisms, which produce products that may be harmful to spermtozoa and which may infect the cow. It is, therefore, standard practice to include antibiotics such as penicillin and streptomycin, in all extenders used commercially.

CHAPTER FIFTEEN

Frozen Semen Technology and Freezing of Semen

India today has less than 9.5 million good dairy cows, about 25 million buffaloes of moderate yield and more than 50 million low producing cows. With this, per capita milk consumption at present is about half the requirement recommended by nutritionists. To meet the national requirement of milk, about 20 million dairy cows will be required Systematic introduction of exotic and improved indigenous germ plasm into the common indigenous cows combined with proper management is the method of choice for increasing milk production.

It has been now realized that for repaid improvement in the milk production, artificial insemination is an indispensible tool. Quality semen, not only in terms of its physical or physiological property to achieve conception, but in term of superior genetic potential, could be used for the large population A bull, if used in natural service can cover about a hundred females in a year. With the artificial insemination technique, using processed liquid semen which can be stored for only 3 to 4 days, about 4,000 to 5,000 cows could be covered by a bull in one year. With the introduction of frozen semen, around 20,000 females, on an average, could be covered by a bull in the same period Thus, it is apparent that frozen semen has the potential to maximize the use of the germ plasm with better production potential which will, in turn, reduce the number of bulls required. Bulls having relatively poor production potential will be automatically culled out.

To evaluate the performance of a bull, the criteria of its pedigree could give information about its production potential. However, to know its actual genetic transmitting ability for a particular trait, the performance of its daughters needs to be known. Frozen semen technology has made it possible to evaluate a bull's efficiency and economics. It has been adequately demonstrated that semen from a bull stored and maintained at extremely low temperature will retain its fertilizing capacity for a long time Thus, after freezing an adequate amount of semen from the bull under evaluation, the bull could be disposed off. The frozen semen of the bull whose daughters show the best performance, could be used for further improvement

With the popularity of frozen semen for routine insemination throughout the world, many attempts have been made to

increase the efficiency of handling, storage and insemination, as well as to reduce the cost involved. The introduction of new packaging methods which increase the storage capacity at ultra low temperature, can play a significant role in this regard.

Efficiency of the semen has been constantly improved since the first calf conceived with frozen semen was born in 1951. From the cumbersome method of slow freezing with alcohol—dry ice bath, instant freezing has been developed for better results. The bulky ampoule, without suitable geometry for good freezing, has given way to a tiny plastic tube which is not only safe and efficient but also reduces the storage requirement, is more convenient for insemination and ensures proper sterilized conditions so vital for its over all success. With the conventional glass pipette method, it is difficult to maintain proper sterilization, specially under field conditions.

Thus, with the introduction of frozen semen packed in straw, the utilization of the quality semen from bulls with better production potential is an answer to rapid prolification of good germ plasm.

Freezing of Semen:

Frozen semen is the extended semen, packaged in a single dose, in a state of suspended animation by deep freezing with virtual suspension of its metabolic activity, without affecting its viability and fertility functions.

Life sustaining chemical processes of spermatozoa are controlled by temperature—higher temperature resulting a short life span. Ultra low temperature preservation of semen, without affecting viability, was possible only after the cryoprotective property of glycerol was discovered in 1947. Since then, considerable improvement in the freezing technology of semen has been going on in the area of proper dilutes and dilutions, cooling, use of packaging material, glycerol mixing equilibration and the other steps of the freezing technique.

Procedure for freezing of semen:

The procedure for freezing varies with the use of the extender, technique of processing and freezing. A rapid improvement in the field of cryopreservation since its accidental discovery in 1947 has taken place. The semen was initially frozen in glass ampoules of one ml or half ml sizes. After filling and sealing these ampoules, they were placed in alcohol dry ice-bath. The temperature was reduced slowly by adding dry ice cubes. The method was very cubersome and was replaced by the instant freezing technique which requires placing the semen in a single dose in the vapours of liquid nitrogen at a temperature of about —196°C. Another instant freezing method with good results was making pellets of processed semen by placing measured small amounts of semen on a block of dry ice. The recovery of the motility however is found suitable but the technique is

not very common as it requires two coolants, namely, dry ice and liquid nitrogen and with this, the semen could not be properly identified. Moreover, the frozen pellet requires a little more skill at the time of thawing, as thawing has to be done in a suitable buffer.

With the introduction of the plastic tube, commonly known as the straw, the freezing of semen has become convenient and more effective in terms of post thaw quality, facility in handling, economy in storage, etc. The straw has been further reduced in size to give better freezing ability and more economy in storage. The capacities of straws available for freezing vary from 0.6 ml to 0.25 ml. These include French-made straw with plastic powder and cotton plug sealing, German straw and Australian staw with glass or metal ball sealing.

The diluters commonly used for freezing vary from egg yolk base to milk base. Various buffers like sodium citrate and tris are commonly used Nowadays, a combination of milk and egg and yolk is used with better results

The most important part of the extender for freezing is glycerol which is the main factor in maintaining the integrity of sperm cell during freezing and thawing. This is known as a cryoprotective agent. Besides, glycerol, other cryoprotective agents like glucose, fructose and lactose are also used in combination on the type of extender to be used for freezing. On an average 50 percent (30-70 percent) spermatozoa are killed during the process of freezing. Therefore, it is essential to choose good quality semen for freezing. A minimum number of 20 million spermatozoa should be kept in the dose. Depending upon sperm concentration and initial motility, the number of doses to be made of a particular ejaculate is decided.

The method of dilution of semen depends upon the extender to be used. In case of extenders with zwitterion buffers like tris, the glycerol can be added to the extender and the semen can be diluted in glycerollated diluent in the beginning only. Even the packaging of the semen in single doses can be performed at room temperature and then the filled straws can be kept for gradual cooling. In case of other diluents, the extender to be used for a particular ejaculate is divided into two equal parts. To one part, semen is added and to the other, the total amount of glycerol required is properly mixed. Both the parts are gradually cooled to 5°C At this temperature, the glycerolled diluent is added to the diluted semen.

The diluted glycerollated and cooled semen is kept at 5°C for at least 4 hours. This period is generally referred as the equilibration time.

The semen is packaged in straws. The straws are printed with the various types of information e.g. number of bull, date of freezing, etc. and are properly sterilized and cooled to 5°C before semen filling. Semen is either filled manually using plastic powder for sealing the other end

or by a filling and sealing machine, using some ways for sealing the end (French straw). Only an automatic or semi-automatic method can be used for filling and sealing (iron ball sealing) German type straws (menitrib).

It is very essential to maintain the filling and sealing machine, straws and other accessories coming in contact with semen at 5°C to avoid any temperature shock to spermatozoa. An air space must be provided inside the straw to take care of expansion and contraction of the fluid while freezing and thawing.

The straws are cleaned of moisture and spread in one layer over racks for horizontal freezing. These racks are kept in the vapours over the liquid nitrogen. The straws are kept for about 10 minutes and then lowered in liquid nitrogen.

It is essential to evaluate the frozen semen at periodic intervals. Frozen semen is thawed in a water bath maintained at 35°C and the motility is observed under a low power microscope. If the motility is found above 35 percent up to one week, the semen is stored for use, otherwise it is discarded.

CHAPTER SIXTEEN

Transport of Semen

Like the seeds of a plant can be taken to different places the artificial insemination technique has made it possible to carry harvested germ plasm of a bull to distant places for insemination of cows. In fact, the huge population of the spermatozoa present in the semen can only be successfully used if a good number of females are available, and this can only be ensured by transporting the semen doses wherever they are required for insemination. Transportation has made it possible to spread the merits of a good bull far and wide.

The number of shipments sent to considerable distances has steadily increased with the recognition of the merits of artificial insemination. The transportation of semen has not remained restricted to nearby places and with the introduction of frozen semen technology, it is possible to carry semen from one country to another. It is now possible to transport and maintain semen at remote places, without any problem.

Transportation of Chilled Semen:

The transport of chilled semen is done in a specially designed insulated box or in a ordinary thermos flask with a wide mouth. Liquid semen transportation should not involve a period of more than 12 to 24 hours in transit, as the semen can maintain proper fertility for 48 hours, after which it starts declining.

It is important to maintain the semen at a temperature of about 5^0C to minimize its metabolic activity and to minimize faster destruction. Ice is used as a coolant and is properly packed in the semen shipper. The amount of ice and the insulation of the shipper should depend upon the ambient temperature. In a hot climate, a larger amount of ice and better insulation is required.

After semen has been properly extended and cooled to 5^0C, it is filled in small pre-cooled vials. The vial is sealed by applying adhesive tape around the stopper. Melted paraffin wax can also be used for sealing. The vial is correctly labelled with necessary information regarding sire number and its breed, date of collection and any other pertinent information required. The vial is wrapped in paper and kept inside a polythene bag. The polythene bag is sealed or properly closed with a rubber band.

The insulation box is properly cleaned and is half packed with ice. The

ice is covered with a thick cotton pad before, putting the vials in it. After the vials have been put in, a thick cotton pad is placed above it. The shipper is properly closed. The correct destination tag should be fixed on the shipper before its despatch.

It is essential to avoid jerks and jolts to the semen shipper during transport. It can be safely carried by road, rail, air or ship. For short distances, motorcycles and bicycles are also used for transportation. It is better to hold a semen shipper in the hand while using a bicycle. Preferably, a small shipper or a one litre thermos flask should be used if the semen is to be transported by bicycle. A smaller shipper can be provided with a shoulder strap so that it can hang on the shoulder to avoid jerks and jolts on a two-wheelers, specially on village roads. A special wooden box with a spring at the base can be used for carrying shippers on rough roads.

Shippers should be kept in a cool place and direct exposure to the sun should be avoided. As most of the shippers are fitted with a double jacketed vaccum bottles, they should not be handled roughly. The ice should be refilled in case it has melted. Care should be taken to avoid any temperature fluctuation of the semen as such fluctuations are detrimental to its fertility.

Frozen Semen:

A proper refrigerant is a must for the preservation of frozen semen to maintain its ultra low temperature. Liquid nitrogen is the refrigerant of choice as nitrogen is an inert gas. The same medium is required for transportation also. However, containers properly evolved to minimize the loss of liquid nitrogen, are required for storage and transport of frozen semen.

Frozen semen is generally transported in small containers. Containers referred to as cryovassels or cryostats are slowly cooled and filled with liquid nitrogen. The liquid nitrogen filled container to be used for transport is kept near the bulk cryovessel from which the semen is to be transported. The canisters are removed and the goblets taken out. The frozen semen is packed in the goblets. Fluctuation of temperature during transport of semen should be avoided. To avoid this, an automatic frozen semen straw counting and packing device, which works in the liquid nitrogen can be used for filling of the goblets. The transfer can otherwise be performed with the help of a properly cooled forceps. While lifting the straw from one goblet and placing in another, the straws should not be lifted more than 4-6 cm above the level of the liquid nitrogen. Both goblets should be submerged in the liquid nitrogen. Even the fluctuations below the freezing point of semen are detrimental to spermatozoa. It must be ensured that the tip of the forceps is properly cooled. This should be done by putting the tips of the forceps in the liquid nitrogen till the boiling of the latter ceases. If the forceps' tips are not properly cooled, it will result

in bursting of the lab plug of the straw. In the case of ampoules, sudden warming can result in bursting of the ampoules.

Protective eye glasses and thick gloves should be used while working with liquid nitrogen. As manual contact and transfer requires extended exposure to nitrogen vapour, the worker should not bend or peep into the container as the higher concentration of nitrogen and low level of oxygen cause giddiness. Longer exposure may cause irritation to the eyes. Direct contact with liquid nitrogen will cause frost bite and should be treated immediately

After the goblets have been filled, they are transferred to the smaller containers required for transport. Before using a container, it should be ensured that the evaporation rate is as per specification and the container is not partly or fully damaged. The goblets are placed in the canisters and the canisters are labelled correctly.

In case the frozen semen is to be transferred from a container with a narrow mouth,, a thermocol box filled with liquid nitrogen is used. The box is filled with liquid nitrogen and covered till the boiling ceases. Goblets from both containers are removed and immediately kept in the thermocol box. The transfer is done with forceps.

The frozen semen container can be transported by road rail and air without any problem. As the liquid nitrogen is in an impressurized state non-explosive, non-poisonous, the regulations allow the liquid nitrogen containers to be transported by all means of transport.

Liquid nitrogen vessels, being highly vaccum insulated require special care, during transportation. Any mishandling may result in damage to the container which will result in fast evaporation of liquid nitrogen and subsequent damage to the semen.

The container should always be kept in an upright position and preferably, in a cool place. Direct exposure to the sun should be avoided. Wooden boxes are also used by some stations for safe transport of these containers. In such cases, enough ventilation should be provided for the escape of the steadily evaporating nitrogen gas. Proper cushioning should be provided to the containers while travelling on uneven and rough roads. While transporting by air, the containers should be kept only in the pressurized cabin of the aircraft.

CHAPTER SEVENTEEN

Inseminating Technique and Handling of Semen

For a successful insemination technique on animals, it is very important to know the details of the reproductive history of the cow or buffalo, as accurately as possible. This should include the age of the cow, number of calvings/lactations, last date of calving, number of previous services, the date of last service and since when the owner has first noticed the heat symptoms. This information will help the inseminator to judge the animal as well as the quality and stage of heat for taking a decision about inseminating the animal. In natural service the bull (male) and the cow (female) decide the most opportune time for mating but in AI the owner decides when to take the cow or the buffalo for insemination

The success of the artificial insemination technique mainly depends upon the following.

1. High quality of semen.
2. Proper care in handling and thawing of the frozen semen.
3. Healthy female in sound breeding condition.
4. Inseminating at the proper time of the estrous cycle.

Semen should be properly handled with great care by the inseminators once it has been handed over to them. Liquid or chilled semen/straws should not be exposed to the sun or wind. To expose semen outside for a long time is harmful to sperms. The straws should be taken out quickly after it has been decided to inseminate the animal. Prime importance should be given for timely replenishment of the liquid nitrogen in the container. A schedule regarding this should be prepared according to the situation and need.

Nitrogen is an inert gas. In it liquid state, it is harmful owing to its extremely low temperature: On evaporation it produces 700 times its volume of gaseous nitrogen, which naturally reduces the oxygen content in the air. So any accidental contact of the skin or eyes with liquid nitrogen (LN_2) or the cold gas, may cause injury similar to burn. Working with LN_2 in a confined area without adequate ventilation can cause asphyxiation. Overflowing, splashing and violent boiling of LN_2 should be avoided. The bowing of the head into the containers for identifying the straw is dangerous Extremely low temperature is likely to cause injury to the conjuctive of the eye. In case of accidental contact with the

fluid, cold water and a cold compress bandage are beneficial.

Frozen semen should be stored continuously at —196°C. It is important that the LN_2 level never runs low. Loss of LN_2 results in damage to sperms even though spermatozoa appear frozen. Great care should be exercised in protecting semen from cold and heat shock.

For a high conception rate the use of frozen semen at the right time of heat is essential. This requires proper detection of estrus and reporting of female in estrus so that insemination is done at right time.

Insemination technique:

It includes following two aspects.

1 *Detection of estrus and optimum time of A.I.* The best indication of estrus is when the female starts mounting other cows or stands when mounted by other male or females. Standing heat is the best time for insemination (Fig. 17.1). Other important signs of estrus include the cow becoming restless and bellowing, and a discharge of clear cervico-vaginal mucus hanging in streak (Buller string). Mucus with reddish streaks is an indication that the estrus occured two to three days earlier and is known as metestrus or post estrus bleeding. This sign can be used to anticipate the next estrus after 16-18 days.

Receptive estrus phase in the female is the best time for insemination. Cows expected to come in heat should be checked twice daily. Inseminating cows in the first half of the 18-24 hours duration of the estrus period, means lower conception rate. Therefore, cows should be inseminated in the latter half of the estrus. As a general rule, a cow seen in heat in the morning hours should be inseminated before the evening on the same day and those cows seen in heat in the afternoon should be inseminated before noon on the next day. About 20 percent of animals do not show the behavioural signs of estrus distinctly and are termed as subestrus or silent heat animals. Cows should not be inseminated before 60 days after calving for best results. Rebreeding sooner after calving requires a higher number of services per conception

Maximum fertility depends on viability and quality of spermatozoa at the time of collection and subsequent

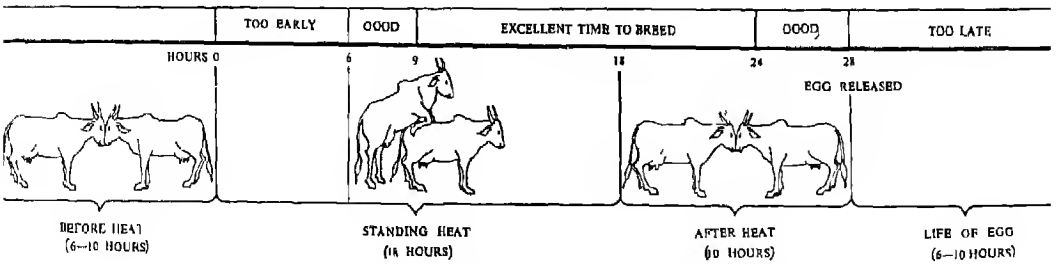

FIG. 17.1 GUIDE FOR BREEDING AN AVERAGE COW

methods used in activation, revival and utilization.

2. *Insemination procedure:* There are two main methods of insemination (i) Recto-vaginal method, (ii) Vaginal speculum method.

Amongst these two methods, the recto-vaginal technique is the most acceptable method and is being practiced throughout the world.

For inseminating with liquid semen, the equipment required includes a glass catheter (glass pipette 40-45 cm long, outside diameter 7 mm and inside diameter 2 mm, with about 1 ml semen not filling more than two-thirds of the length of the glass pipette) and a syringe. One end of the catheter is tapered and to the other end a 2 ml hypodermic syringe is attached with a rubber adopter or connector. The syringe is used to draw the semen into the catheter and also to expel it at the time of actual insemination. The cow can be inseminated while standing in the stall. An insemination crate can also be used to restrain the individual animal.

(i) *Recto-vaginal method:* [*a*] *Insemination with liquid* [*chilled*] *semen:* The recto-vaginal method is almost exclusively used for inseminating cows and buffaloes (*Fig. 17.2*) After having thoroughly cleaned the externally visible genitalia, one hand is lubricated and is introduced into the rectum. The cervix is

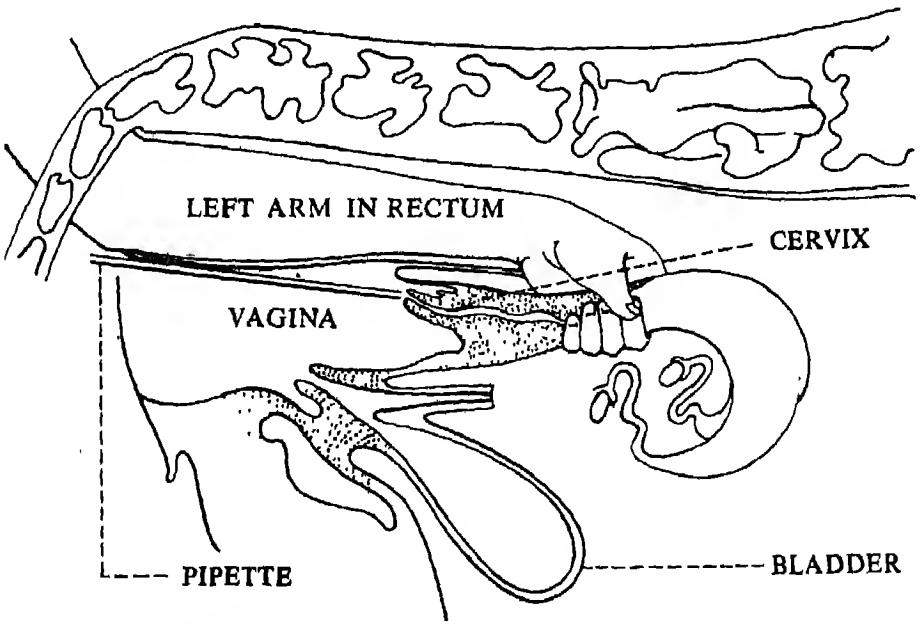

FIG. 17.2 THE RECTOVAGINAL METHOD FOR CERVICAL INSEMINATION THE COW.

located through the rectal wall and the female reproduction tract is manipulated and examined If the animal does not present any abnormality, the steps for insemination are undertaken. This consists of gripping the cervix and locating the OS cervix with the help of the thumb. Now the sterilized glass pipette containing semen is introduced through the vulva and vagina into the external OS of the cervix By manipulating the cervix and the pipette, the inseminating catheter is worked through the cervix so that the tip of the pipette is located in two-thirds length of the cervix. The semen is deposited in the region by pushing the syringe piston. After depositing the semen the pipette is withdrawn carefully.

(b) *Insemination with frozen semen:* Insemination with frozen semen is carried out with the help of an inseminating gun. Once it is decided to inseminate the animal, a straw is taken out from the LN_2 container, then thawed and loaded into the AI gun. The straw (13.5 cm in length and 3 mm outside diameter) is made of polyvinyle chloride. The steps involved in insemination are as under:

—Lift the neck tube plug of the LN_2 container straight upwards to open the container.

—Cool the tip of the forceps by holding it in the vapours of LN_2 near by the neck of the container for a few minutes

—Lift the canister by its handle so as to clear the neck ring.

—Do not lift the canister more than the bottom level of the neck; lifting above will cause a sudden rise in temperature.

—Pick-up one straw with the forceps, taking care not to touch other straws unnecessarily

—Replace canister and plug top quickly and carefully.

—Shake the straw once/twice to remove LN_2 drops.

—Plunge the straw into a water bath at 20-24°C for thawing. Preferably, in field conditions 35°C ± 2 temperature for one to two minutes (thawing).

—Take out the straw from the water bath and dry it carefully with a clean towel.

—Hold the straw at the single sealed end, shake it to bring the air bubble near the single sealed end.

—Load the straw into the chamber of the AI gun so as to keep the laboratory seal at the tip of gun.

—Slip the sheath over the entire gun and secure it tightly with the ring provided along with the equipment.

—Inseminate the animal as detailed in the procedure for insemination with chilled semen.

It is necessary to note that the sperm do not survive for a long time after thawing. Therefore, the thawed straw of frozen semen should be used immediately. This specific procedure must be followed

for obtaining optimum fertility To avoid the crystallization zone, the thawing should be rapid. The frozen semen needs to be thawed evenly and progressively. The method employed is to plunge the straw into water. The time allowed depends on the temperature of water and the packing system (ampoules, straw, pellet). The pellets are best thawed by transferring to a liquid thaw media.

(ii) *Vaginal Speculum Method:* In the speculum method, a vaginal speculum, sterilized and lubricated, is introduced through the vagina to locate the OS uterus. The inseminating catheter is then introduced through the speculum into the cervix where the semen is deposited.

Advantages of the recto-vaginal method (RVM) over vaginal speculum method (VSM):

—The RVM method enables the examination of the reproductive tract and diagnosis of abnormality.

—Through the RVM method pregnancy can be diagnosed so that insemination of a pregnant animal is eliminated

—The semen can be accurately deposited at the proper site through manipulation through the rectum in the RVM.

—The RVM obviates the use of speculum and the elaborate process of its sterilization.

—There is no chance of infection being carried through the unsterilized equipment if the RVM is followed. In the speculum method, to open the reproductive tract a sterile vaginal speculum is used to locate the cervix and the insemination is then performed

Precautions to be taken during Insemination:

The following precautions are required to be taken while performing insemination

—Identify the female in estrus.

—External genitalia should be thoroughly cleaned before passing pipette/gun through vulva and vagina

—Care should be taken to avoid entrance of the pipette/gun into urethra/suburethra/diverticulum.

—Insemination should be intracervix rather than depositing the semen intracornually or into the vagina.

—Only sterilized insemination pipettes, rubber junction and syringe or gun and sheaths are used.

—No part of the glass pipette or of the gun, covered with sheath which comes in contact with the vagina should be touched by hand or clothes.

—While handling the gun, the factory seal of the semen straw should be down and the laboratory seal should be at the top.

—Semen straw should be cut at a

90° angle to avoid back flow of the semen into the sheath.

—A minimum of 10-12 million of actively motile spermatozoa should be inseminated for better conception rate.

—Frozen semen that is used for insemination should have 40 percent or more motile spermatozoa.

—There is a tendency of estrus to occur in the first three months of pregnancy (gestational estrus); great care should be exercised in taking into account the previous date of insemination, the intervals and the avoidance of passing the pipette deep into the uterine cavity

—Proper records of the inseminated animal must be maintained. It should be ensured that the owners are given an individual AI card for each female, which carries all the information regarding the reproductive status of the animal

—The insemination work is to be carried out with least disturbance to the animal

—When animals have travelled long distances (more than 3-4 km) for the insemination, they should be allowed to rest before the insemination is carried out.

It can be seen from the detailed description given above regarding the insemination technique and care and handling of semen, that the success of the operation depends on how correctly the female in heat is detected at the right stage of estrus and the accuracy and skill with which the semen is placed at the right site. The potential of AI depends on the combined skill of insemination, thoroughness and perfection on the part of the organization producing the semen and distributing it and the cooperation from animal owners

CHAPTER EIGHTEEN

Recent Biotechniques in Animal Reproduction

Production of off spring requires a high degree of synchronization of very complicated processes, in both the male and female. Thus, it is not surprising, that only about 50 percent of the cows deliver a normal calf from the first breeding/insemination. Although infertility is a major problem for our animal improvement programmes, enough basic information on reproductive physiology is available so that some of these losses can be reduced. New techniques have been developed during the last decade which have revolutionized the process of what used to be a male and female union The new techniques and the animal biological systems have not only increased the use of superior individual animals but have also brought in the latest technology for fertility improvement, breed development and augmentation of reproduction Some of the major bio-techniques are discussed below:

1. Artificial Insemination (AI):

A.I. is the most important single technique ever devised for the genetic improvement of animals. This is possible because highly selected males produce enough spermatozoa to inseminate thousands of females in a year Carefully selected young dairy bulls, when subjected to progeny testing, help early evaluation of the young sire and, thus, also help in measuring and enhansing productivity. This biotechnique includes management of the male, semen collection and evaluation and its preservation through the use of a number of diluters or extenders.

2. Processing of Semen and Frozen Semen Technology:

The processing of semen through cooling it to 5°C is similar whether it is to be used frozen or chilled For preserving semen for long periods, the technique of deep freezing of spermatozoa, already extended, has been developed. Glycerol is used almost universally as a cryoprotective agent for freezing semen and storing the same at a temperature of—196°C. Semen was initially frozen in glass ampoules, each sufficient for one insemination but now polyvinyl chloride straws have been developed. They require less space for storage and have better freezing characteristics and can be labelled, filled and sealed automatically. Also, semen can be inseminated with minimum loss of

sperm cells Through this technique valuable semen with high potential can be stored for decades for use as and when required.

3. Estrus Detection:

Certain hormone assays using immunological principles for detecting very low quantities of hormone have been developed. These mocro-quantitation immunoassay methods have helped in identifying the estrus in animals. Hormonal pheromones are also currently being put to test as an efficient estrus detection tool

4. Synchronization of Estrus

The possibility of inducing estrus and ovulation in acyclic females and synchronization of estrus and ovulation in groups of females have offered an opportunity to increase the efficiency of animal production and to increase application of A.I. With these methods, initial conception can occur at an early age and the interval between successive pregnancies can be reduced These methods have been developed, on the basis of the knowledge in the area of endocrinology, and use of these hormones on living animal systems have been achieved. The two major techniques employed for getting a group of animals to cycle together or show estrus together consist either (i) in increasing the life of C L. through the use of long term progesterone administration or, (ii) by shortening the life of CL through the administration of luteolytic compounds like prostaglandins.

5. Induction of Ovulation:

In order to increase ovulation in anestrus and acyclic females, a single follicle or a group of follicles must be stimulated to develop to a state of maturity so that a surge of LH or a hormone with LH like properties such as Human Chorionic Gonadotrophin Protein (HCGP) will cause ovulation. This can, therefore, be done by using Gonadotrophins or Gonadotrophin Releasing Hormone (GnRH).

In sheep, cattle and goats, gonadotrophin treatment does not normally stimulate behavioural estrus even though follicular growth and ovulation is seen. For AI, the animal must exhibit estrus Thus, an ovulation induction regime in these animals should include steroid hormone i.e. progesterone treatment, for a period of time to bring about behavioural estrus. Gonadal steroids or their synthetic counterparts have been used for this purpose. Often, a combination of estrogenal and progestational compounds are used.

6. Induction of Parturition:

Hormonal compounds have been used for early termination of pregnancy These compounds include two major groups, namely, corticoids and prostaglandins Inducing parturition is particularly useful as a managemental aid in preventing what would otherwise be late calving and in enabling the cow's breed-

ing pattern to be brought in line with that of the main herd for the subsequent calving season. It is of great field applicability in clinical cases of maternal ill-health, particularly cervico-vaginal prolapse, a condition often seen among buffaloes during the last month of pregnancy It can also be used as a means of ensuring calf viability by lowering the level of perinatal mortality Under practical conditions, 25-40 mg of dexamethasone for cattle, 30-40 mg for buffaloes and 20 mg for goats have been successfully used for inducing parturition as early as 30 days without affecting the calf survival, milk production, post-partum reproductivity and milk composition.

7. Induction of Lactation;

Lactation among cycling infertile animals has been successfully induced by hormone administration. These animals, receiving a combination of estrogen and progesterone through seven injections over a 2-week treatment regime, are brought into milk. The animal produces as much of milk as per her genetic capacity. This method has been found to be very useful among crossbred females and animals with high milk potential on becoming repeat breeders or infertile. Consequent to the short hormone treatment, animals go through a full length lactation, producing as much milk or even more than what they had produced during the previous lactation.

8. Pregnancy Diagnosis:

Besides the clinical methods of pregnancy diagnosis which include rectal examination radiography and ultrasonic techniques, tests like vaginal biopsy and hormonal assays have been developed. Histological changes that occur in vaginal epithelium during pregnancy form the basis of pregnancy tests in farm animals Numerous vaginal biopsy instruments have been designed. Biopsy samples are subjected to routine histological procedures

Biological and chemical methods are employed for the detection of pregnancy-dependent hormones in body fluids. The protein hormones PMSG or HCG are tested through bioassay for early pregnancy in some species while chemical and immunochemical tests for steroids, like progesterone have found universal application These tests are based on the quantitation of circulating levels of hormones in plasma or milk

High post-insemination levels of progesterone by day 21 suggests that the pregnancy could be positive. A close relationship exists between progesterone levels in plasma and in the milk of lactating animals. Thus, even milk samples have been successfully used for detection of pregnancy A plasma level of less than 0.5 ng/ml (nanogram) and a milk level of less than 2 0 ng/ml is indicative of the animal not being pregnant

9. Superovulation:

The objective of superovulation is to increase the yield of viable ova. Usually, subcutaneous (s/c) or intra-muscular

(i/m) injections of PMSG or FSH are given to stimulate additional follicular growth. This is followed by i/v injection of LH or HCG, several days later, to induce ovulation in a large number of follicles which have matured as a result of the first injection. With current procedures, superovulation increases the yield of normal embryos above five to eight fold in cows, though there is tremendous individual variation in response. The greatest advantage in superovulation methodology in the last decade has been the use of prostaglandin F_2 alpha (PGF_2 alpha) analogues. Their use has brought about flexibility of timing superovulation and is also an excellent method of obtaining a large number of normal embryos.

10. Embryo Collection and Transfer:
Embryos can be collected from oviducts or uterus either through surgical or non-surgical methods. Embryos reside in the oviducts for three to four days. Thus, flushing the oviduct one to three days after estrus yields a greater number of embryos than flushing the uterus five or more days after estrus. However uterine embryos are often collected because they result in higher pregnancy rates and can be frozen more successfully than younger embryos.

Currently, among bovines, the non-surgical method is the technique of choice. Foley catheters, (three way) or similar catheters are used for non-surgical embryo recovery. The cervix of the animal is dilated. Each uterine horn is filled with 30-60 ml of buffer medium through a catheter which is held in position in the uterus by the air bulb. The fluid is then collected in a vessel while the uterus is gently massaged per rectum.

After collection, embryos are examined under a microscope and only morphologically normal one are transferred. The stage of embryonic development can be observed when the embryos are examined microscopically. Generally, embryos of fewer than eight cells should be transferred to the oviduct and embryos with more than eight cells into the uterine horn. Though both surgical and non-surgical methods of embryo transfer are available, again the method of choice is non-surgical The embryo is deposited in the uterus through the cervix with an A.I. gun 6-8 days after estrus.

For effective transfer, there should be a thorough synchronization of estrus between donor and recipient. For optimal results, the recipient should be in estrus within 12 hours of the donor. The pregnancy rate decreases drastically if the difference is greater than 24 hours in cows.

11. Freezing of Embryos:
For ordinary handling and manipulation, the embryos are kept in a culture medium and can be held for several hours at 15-25°C. For long term storage and easy transportation, embryos in late-morula or early blastocyst stage may also be frozen to liquid nitrogen temperatures. This technology of freezing

embryos has had rapid development during the past few years. All the principles that apply to freezing any living cell, apply to freezing embryos also. Cryoprotectants, such as glycerol, are used in this process. Embryos are cooled rapidly to 0^0C and at a rate of 1^0C/minute to -7^0C, at which point freezing is initiated by adding a small crystal of ice to the medium. Embryos are then cooled very slowly (0.3^0C /min) to about -33^0C and finally placed in liquid nitrogen for storage for years.

12. In-vitro Fertilization, Cloning and Sexing of Embryos:

Current technology has introduced in vitro fertilization which was so far used mostly for research purposes only. In the near future, it will have also application in the culturing of follicular ova from slaughter house ovaries, testing of male fertility, sexing of embryos, and cloning of embryos.

Though these technologies can usher greater productivity from animals, they also demand better understanding of animal physiological functions and knowledge of the animal's reproductive processes.

CHAPTER NINETEEN

Techniques in Embryo Transfer Technology

Embryo transfer is a specialized technique of breeding. A sexually mature female, referred to as the donor, is injected with exogenous hormones to produce more ova, which are fertilized inside her either by natural or artificial service. These are then removed prior to their implantation and transfer to the reproductive tracts of synchronized surrogate mothers of the same species referred to as the recipients The fertilized ova, thus are developed in the recipient body and the resulting off spring derive their genes from the donor and from the male to which the donor was bred.

The embryo transfer technique consists of several important steps, such as:

1. Selection and management of donors and recipients.
2. Superovulation and estrus synchronization of the donor and the recipients.
3. Insemination of the donor.
4. Collection of embryos.
5. Identification and storage of embryos
6. Transfer of embryos.

Donors should be selected according to their genetic superiority and reproductive efficiency In dairy cattle, selection should be based on production records and progeny tests. In selecting recipients, factors such as reproductive soundness and body size should be taken into consideration to obtain high conception rates and avoid problems of dystocia.

Superovulation is a technique in which the female donor is injected with exogenous hormones to induce multiple ovulations. The hormones used are pregnant mare serum gonadotrophin (PMSG) and follicle stimulating hormone (FSH). The usual dose of PMSG to superovulate a cow is 2000 IU in a single injection and that of FSH is 30-50 mg, each in 4-5 injections. Prostaglandin F_2 alpha either in synthetic form (500 mg) or in natural (25 mg) form, is injected to the donor and the recipients to synchronize their estrous cycles.

As ovulation in superovulatory cows is spread out over 24-48 hours, they should be inseminated more than once. The time of insemination and the number of doses of semen depend upon the time of onset of estrus, duration of estrus and quality of semen.

Usually, embryos are collected 6-8 days after the onset of estrus. The standard procedure in most embryo transfer

centres for the non surgical collection of embryos is as follows:

The donor cow is given epidural anaesthesia with 5-6 ml of 2 percent xylocaine. The perineal region and vulval lips are washed thoroughly and disinfected A gloved arm is introduced into the rectum to locate the reproductive tract. The ovarian response on both ovaries is ascertained and noted. Then, a sterile steel rod (2 5 cm diameter) is introduced by everting the vulval lips for dilating the cervix. The cervical dilator is withdrawn and a sterile foley catheter with a sterile stillette is introduced gently through the cervix and into one of the uterine horns.

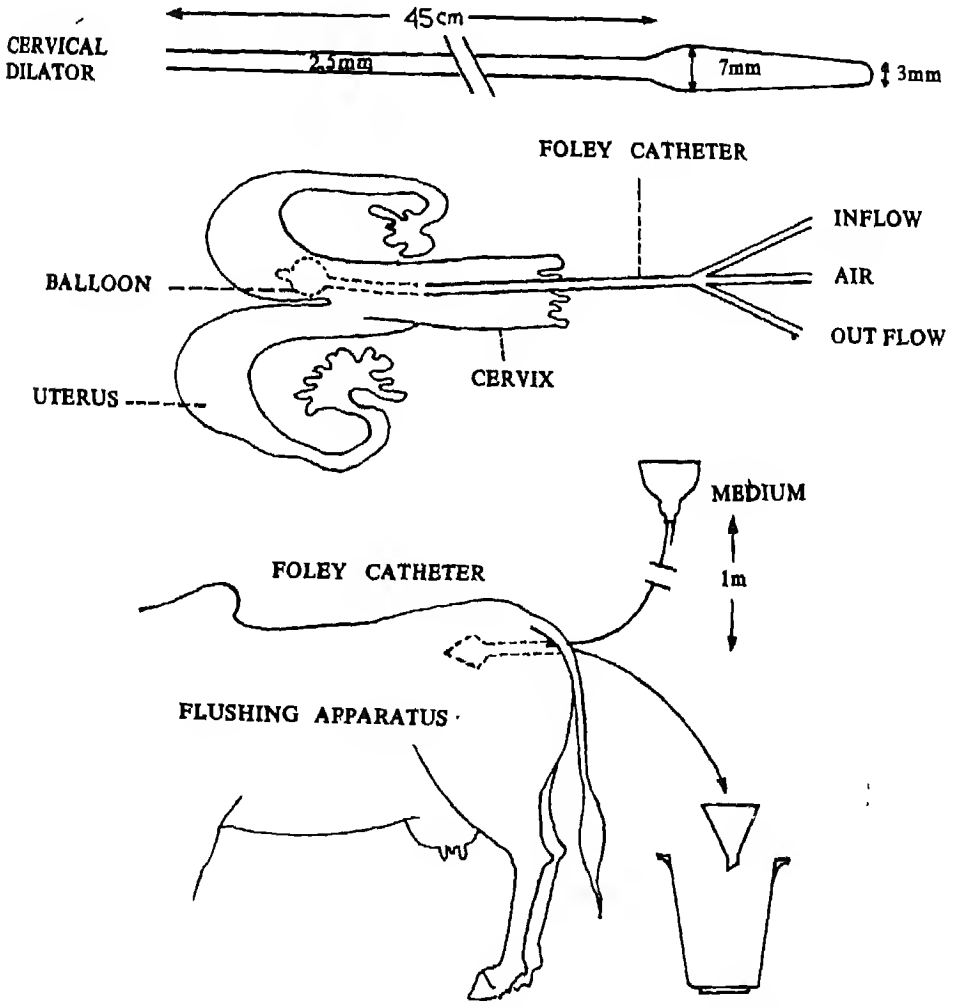

FIG. 19.1 NONSURGICAL RECOVERY OF BOVINE OVA USING CERVICAL DILATOR AND FOLEY CATHETER

When the cuff of the catheter is about 2-3 cm anterior to the biturcation, it is inflated with about 10-15 ml air depending upon the size of the uterus The stillette is withdrawn gently and the catheter connected to a Y junction which has inlet and outlet tubes already connected to the bottle containing the medium (phosphate buffered saline enriched with 2 percent fetal calf serum and antibiotics at the rate of 100 IU of penicillin, 100 mg of streptomycin and 0.25 mg of amphoterisin-B per millilitre of solution) by means of a special needle. Both inlet and outlet tubes are clamped with two pairs of artery forceps. The bottle containing the flushing medium is elevated to about 3 m from ground level. About 100-150 ml of medium is allowed to flow into the uterine horn by unclamping the inlet. The uterine horn is held in the palm of the hand in the rectum as the fluid enters the uterus. When it attains the size of 6-8 week pregnancy, the inlet is clamped and the outlet unclamped. The uterine horn is massaged gently to empty it of the medium. When the uterine horn is nearly empty, the outlet is clamped and another 100-150 ml medium is allowed into the uterus, by unclamping the inlet The same procedure of massaging and emptying the uterine horn is repeated. Thus, the uterine horn is flushed with about 500 ml of medium. After flushing one horn, the catheter is withdrawn and the same procedure is followed to flush the opposite horn using a fresh catheter. After flushing both uterine horns, an antibiotic solution containing penicillin and streptomycin is infused into the uterus. (*Fig 19.1*)

The final and most important step in embryo transfer technology is the successful transfer of the embryo into synchronized recipient. While selecting recipients, preference should be given to those with 'O' synchronization. Basically there are two methods of transfering embryos in cattle; the surgical and non-surgical methods

In the surgical method, the recipient is palpated to identify the corpus luteum. If the corpus luteum is on the right ovary, the right flank is prepared for surgery and vice versa Then, the animal is given local anaesthesia and the ipsilateral horn exteriorized through a laporotomy incision in the flank. The embryo is transferred close to the tip of the uterine horn either with a pasteur pipette or a micropipette. Pregnancy rates ranging from 50-70 percent have been reported by several workers

In the non-surgical method, the embryo is aspirated into either a 0.25 ml or 0 5 ml straw in such a way that it is locked between two air bubbles. Then, the A.I. gun is loaded, as in the case of the artificial insemination technique Epidural anaesthesia is given to the recipient and the ipsilateral horn located by palpation of the ovaries The vulva and perineal regions are cleaned and disinfected. The A I gun is introduced through the cervix into the middle of the ipsilateral horn and the embryo is deposited gently. Pregnancy rates reported from this technique vary from 40 to 70 percent.

CHAPTER TWENTY

Cleaning and Sterilization of A.I. Equipment

Artificial insemination requires handling of the male germ plasm from the stage of its collection from a bull to the stage of its insemination in the cow. It comes in contact with various collection equipment, lab-wares, buffers, extenders, packaging material, insemination devices, etc. If the equipment is not properly cleaned and made free of microorganisms, it may result introducing infection in the female reproductive tract. The presence of micro-organisms in the semen may effect spermatozoa directly, even if of nonpathogenic nature, as they may compete for substrater in the extender. The microorganisms may also infect the female with a resultant lowered conception rate, increased embryonic mortality and abortion. Therefore, scrupulous cleaning and sterilization is a must for all equipment and buffers before they are used in the process.

Various types of materials, viz. glass, rubber, different types of plastics and solutions are used in the semen collection, preservation and insemination procedures. Each type of material requires a specific type of cleaning and sterilization. A variety of detergents for cleaning are available in the market. Thus, a thorough selection of these is necessary specially for A.I. equipment.

Selection of Detergent:

It is necessary to choose a good detergent which should have no free alkali, should soften water, is easily soluble in water, does not precipitate in hot water and cold water, rinses very easily, has lower surface tension, has good wetting ability and is non-irritating to the skin. Some of the common detergents are as under:

—Soft soap is quite suitable for the purpose, but soap powders available are not suitable as they cause cloudiness

—Washing soda

—Vim powder

—Sodium hexa-meta-phosphate—0.2 to 0.3 percent

—Tetra-sodium pyro-phosphate—0.3 to 0.5 percent

Cleaning and Neutralization of New Glassware:

Cleaning: Newly purchased glassware required special attention because of existing spores of various bacteria in straw, hay and other packing materials.

Thorough cleaning with hot water, soap and brush is not sufficient. It is essential to boil the glassware for 15 minutes in 2 percent washing soda solution or one hour in 5 percent soft soap solution of 3 percent lysol solution.

Neutralization: The cheap variety of glass gives off free alkali and therefore a change in pH is affected. It is always advisable to purchase chemically clean and sterile glassware from reliable firms. It may be necessary to place cheap variety of glassware in 1 percent hydrochloric acid solution for 2 hours for neutralization. After this, it is necessary to rinse well with tap water.

Cleaning and Sterilization of Used Laboratory Glassware:

Cleaning: Cleaning should be done as soon as possible after use. It is necessary to empty the contents of the glassware to be cleaned and then soak it in 2 percent washing soda solution which facilitates the cleaning work Scrubbing with a brush (depending on the type of article, use test-tube brush, burette brush, etc.) and soap water, internally and externally is absolutely necessary for complete cleaning. A worn out brush should never be used as it can cause markings on the glass and the to-and-fro movements of the brush in the test-tube can break bottem of the tube Special attention should be paid to inseminating and graduated pipettes as the lumen is small. Prior to their cleaning, it is necessary to keep the used up pipettes immersed in a jar or cylinder, with cotton wool pad and water, preferably in a sodium carbonate solution.

Clouding of glassware: Free use of *bazar* soap powder causes clouding of glassware. If the water used for washing is hard, deposition of salts of Ca and Mg remain on the glass. Cloudy glassware can be treated for 24 hours with any one of the following mixtures which need to be prepared carefully.

1. Potassium dichromate 2 parts, commercial sulphuric acid (H_2SO_4) 3 parts, Water 25 parts.
2. Potassium dichromate solution prepared as above (20 ml), Sulphuric acid C.P grade (20 ml), Water (200 ml).
3. Potassium dichromate 10 percent solution.
4. Sulphuric acid solution 20 percent.
5. Nitric acid 6 parts, Potassium bicarbonate 6 parts, Water 100 parts
6. Potassium dichromate 50-100 g, Conc hydrochloric acid (HCl) 1000 ml.

All the glassware should be rinsed in any one of the above solution for 24 hours and then washed thoroughly for final cleaning.

Rinsing: After brushing, rinsing of glassware in running water is necessary. Later, it should be rinsed with distilled water. In case of scarcity of distilled water, only the internal side of the glassware may be taken up.

Draining: This is done by inverting the test-tubes in a wire basket and other articles like flasks, etc., can be hung on wash boards having slanting pegs.

Drying: Rinsing in hot water is said to facilitate drying of the glassware. An electrical contrivance used for quick drying of the glassware can be used.

Plugging of glassware: Before sterilization, all glassware will have to be plugged with non-absorbant cotton wool stoppers and covered with kraft or brown paper, etc. As it is necessary to maintain absolute sterility of the apparatus after sterilization, it will have to be kept away from access to air, dust and contamination. Long fibre cotton of non-absorbant nature is necessary for plugging. Cotton wool can be forced with a rod or forceps but never twisted, as crevices are formed and these make way for organisms. Cotton wool in the form of roll is used for plugging.

Precautions:

—Glassware must be perfectly dry.

—Oven must be cool when fresh apparatus is inserted and then heated to the requisite temperature to be maintained for the period necessary for the purpose.

—The oven must be allowed to cool before the articles are removed.

Sterilization:

This is a method by which all forms of microorganisms are killed. The method of sterilization varies according to the nature of the article to be sterilized. Each method has its own use with limitations and certain advantages.

Dry heat:

This requires higher temperature and less time than moist heat. Most resistant forms of microbial life are not easily killed by dry heat or chemicals, but by moist heat at 100°C they are easily killed in few seconds. Some spores survive up to 90 minutes.

(i) *Direct heat:* Bunsen burner flame or spirit lamp flame (direct heat) is used for sterilization of inoculation needles and points of forceps and scalpels, etc.

(ii) *Indirect heat:* A hot air oven is thermostatically controlled and consists of a strong chamber made of a metal welded cabinet having double walls between which air passes. It is electrically operated with uniform current and voltage. The large working chamber has a durable aluminium or spray enamel finish or stainless steel. It has an accurate thermostat arrangement with sealed heavy glasswool blanket insulation, braided asbestos door gaskets, having

safe tight closing latch and adjustable ventilating shutter. It has nickel chromium heating elements, massive heavy hinges, a knob for thermostat setting and signal light, with arrangement to fix a thermometer. It can be operated from the mains 200-250 volts AC single phase. The temperature is controlled in the range 30° to 180°C with accuracy .5°C. The oven (indirect heat) is used for sterilizing dry glassware like test-tubes, petri-dishes, flasks, graduated pipettes, cylinders, all glass syringes, inseminating and graduated pipettes, drawn out pipettes, beakers, etc For practical purposes, the time required for sterilization of A.I equipment at 140°C is one hour.

Moist heat:

This requires water and high temperatures. Heating is done for a longer time.

(i) *Instrument sterilizer:* An electric instrument sterilizer (size 20" x 8" x 6") can be used for sterilization of the instruments. It is made of heavy bronze cast, with the body covered with stainless steel. It has a tray which can be automatically lifted along with the cover with a lever handle It has a pilot light and lining cord to work on AC and DC 220 volts. (Temperature 100° C for ½ to 1 hour) This is used for a sterilizing instruments, pipettes, forceps, scissors, speculums, etc. If the water is hard, it is better to use distilled water for boiling purpose

(ii) *Autoclave:* This is used for sterilizing surgical dressings, glass containers, buffers, solutions, pathogenic cultures, etc. Glassware must be packed and sterilized usually at 15 lb pressure for 15 minutes (125°). Effective sterilization in the autoclave depends upon the temperature obtained and not directly upon pressure. The pressure temperature relation applies only if the sterilizer chamber is kept free of air during the period in which steam enters the chamber. This can be accomplished by keeping the chamber drain valve slightly but continuously open. If air remains in the chamber, the temperature actually obtained is much lower than that indicated and sterilization will be entirely ineffective:

Precautions:

- —There must be sufficient water in the cylinder (about 7.5 cm).
- —Keep material in such a way that there is not much agitation of the flasks.
- —Allow the autoclave to cool before removing the contents.
- —It is necessary that the flasks are stoppered with non-absorbant cotton wool and gauze.
- —Parchment paper or kraft paper should be used to avoid drenching of cotton wool stoppers.

Sterilization of Rubberware:

The Artificial Vagina (A V.) is one of important rubberware. It is thoroughly

rinsed after use under tap water to remove traces of dung and dirt. The cone is washed separately to remove traces of semen, using warm, soapy water and a sponge brush, followed by rinsing with tap water to remove all traces of soap and double distilled water to remove traces of tap water. The A.V is then placed in the A V sterilizer, which consists of a water bath with heating elements and enough space for the A.V to be kept above the level of the water. The water is brought to boiling point and the A V. is steamed for about 15 minutes. The A.V. is removed, dried and dipped in 60-70 percent ethyl or isopropyl alcohol. This should be applied at least for 15 minutes The A.V. is finally stored in a dust-proof cupboard.

Rubber stoppers, rubber tubing, etc. are left in alcohol for at least one hour after thorough washing and rinsing. Most of the rubber/plastic tubing used in processing of semen for freezing is, thus, discarded after use.

Sterilization of Plasticwares:

Most of the plasticware e.g. straws, sheaths, insemination pipettes, filling combs, etc. are presterilized and used only once However, these items are stored for a long time, and after opening a packet it may not be used immediately, hence. it is essential to sterilize these articles immediately before use. The most suitable way of sterilizing these prepacked disposable items that are unable to withstand heat, is by the use of radiation Ultra voilet radiation can be produced artificially by mercury vapour lamps (240-280 mu) and is most effective in the range.

The items are spread in a thin layer and are exposed to radiation for at least half an hour The exposure should be allowed from all sides of the material packaged in polythene bags as the penetration power of ultra voilet rays is limited. However, exposure of the person handling the items should be avoided The radiation should be given in a closed cabinet to avoid any exposure of the workers to radiation.

Sterilization of Buffers:

The best method for sterilization of aqueous solutions is exposure of the solution to heat sufficient for sterilization but not so excessive or prolonged as to damage the heat sensitive components of the buffer. This is achieved by autoclaving or keeping the solution in super-heated steam, created by applying pressure to it, using a pressure steam chamber known as an autoclave.

The buffers to be used are prepared in a glass flask with double distilled water and the flask is stoppered with a cotton plug before keeping it in the autoclave. The lid of the autoclave is opened It must be ensured that there is adequate water inside to generate enough steam and that it does not get dry which may result in damage to the heating system The water level should be below the level

of the basket in which the flask is kept. The autoclave lid is closed firmly after placing the flask, and the power is switched on. After the air inside has been pushed out by steam, and steam starts coming out, the steam valve is closed and pressure allowed to reach 15 pounds per square inch. This pressure is to be maintained for at least 15 minutes. This can be done by fixing the pressure guage and timer provided with the autoclave, or manually.

Maintenance of Sterility:

It is very essential to maintain the items sterilized in the same condition till they are used This can be achieved by keeping various articles in dust-proof enclosures. The glassware can be stoppered with cotton wool before sterilization and wrapped in paper which should be removed only at the time of use. The buffers should be stored in the refrigerator, properly stoppered. Plastic ware should be kept packed in polythene bags and should be removed only at the time of use.

The workers should have clean habits They should be aware of the importance of cleanliness while working in an artificial insemination laboratory. Animal attendants working outside the laboratory should not have direct access to the semen laboratory without changing aprons. One should never enter the laboratory wearing the same shoes as during semen collection and insemination.

Fumigation of the laboratory can also be carried out at the weekend to make the atmosphere sterile.

PART III

REPRODUCTIVE MANAGEMENT

CHAPTER TWENTY ONE

Organization of Artificial Insemination Laboratory and Its Basic Equipment

The bull station-semen bank complex is the nucleus of the artificial insemination organization. Sometimes, it is a part of a farm, such as the bull farm or dairy farm. Therefore, it should primarily satisfy the requirement of the field and at the same time function well in combination with other enterprises of the farm to which it is attached. The planning of the AI lab should be so regulated that it has the necessary number of bulls and is located in a place where its functional requirements and other resources are available.

For the establishment of an AI complex there should be two component viz bull station and semen collection and processing units. The requirement of the bull station depends mainly upon the requirement of semen, both for local use as well as for supplying to out-stations Whatever be the requirement of semen, in terms of relative economy of the investment, it is not advisable to plan a bull station with less than 40-50 bulls

Location of the Buildings:

A laboratory-bull shed complex should be located at a higher ground level and in such a way that it is isolated from other functions of the farm (like fodder farm, animal sheds, administrative wing).

Bull Station· The housing arrangement for bulls should be hygienic, comfortable and inexpensive. For each bull, there should be a separate loose box of 3.70x3.70m with a height of 2 5 to 3m and a run of 8x6m. Feeding manger and water troughs should be included in the bull-paddock and should have provision for good ventilation and light

Two single row sheds for bulls, each with an adjoining store are always found easy and trouble free There should also be provision for segregation of sick bulls

Semen Collection and Processing Units:

(1) *Semen collection shed.* The semen collection shed should be near the bull-paddock and it should be free from dust and direct sunlight and protected from summer and winter winds. A floor space of 8x9 m can house two service crates. One for the buffalo and other for the cow bull The service crate should be prepared from wood or galvanised iron pipes and

for the buffalo bull it should be wider in size and should have a platform on either side for support to the forelegs. The floor of the shed should be made of bricks or non-slippery concrete

(ii) *Semen processing laboratory:* The semen evaluation and processing laboratory should be near the collection shed so that semen evaluation and processing can be carried out without any loss of time It should be hygienic and dust proof and provided with electricity and water connections and the room temperature should be maintained at 22° C. It should have the following rooms· (a) A.V. Room (b) Change Room (c) Sterilization Room (d) Processing and Evaluation Room (e) Freezing Room (f) Semen Storage Chamber and Liquid Nitrogen Storage Room (g) Liquid Nitrogen Plant Room.

All these rooms should have enough light, furniture and hygienic conditions and should be easy to clean and disinfect

The following is a complete list of all essential instruments and equipment for establishing and operating an AI laboratory including the field insemination unit:

A. Rubber, Glassware and Plastics

S. No	*Name of Article*	*No required*
1.	Artificial vagina (Swedish model 14" and 12" long with air and water valve fitting)	6
2.	Latex liners for artificial vagina (smooth)	18
3.	Latex liners for artificial vagina (rough)	18
4.	Latex collecting cones (long to fit above model A V.)	18
5.	Insulating bag for collecting tube	2
6.	Graduated semen collecting tubes (graduated centrifuge tubes 15 ml)	24
7	Mortar and pestle (glass 4" diameter)	1
8	Glass rods for lubricating vaginas (1" diameter)	4
9.	Graduated measuring cylinders 25 ml with snout	12
10.	Graduated measuring cylinders 100 ml with snout	6
11.	Graduated measuring cylinders 500 ml with snout	2
12.	Beakers (Pyrex) 500 ml capacity	12
13.	Beakers (Pyrex) 250 ml capacity	6
14.	Flat bottomed flasks (Pyrex) 500 ml	6
15	Flat bottomed flasks (Pyrex) 5 litres	2
16	Glass rods 3 to 4 mm diameter made of soft glass	1 kg
17.	Test-tube rimless 1 cm dia 3" long	2 gross
18.	Reagent bottles, wide mouth, with dust-proof stoppers 1000 ml	2 dozen
19.	Reagent bottles, wide mouth, with dust-proof stoppers 500 ml	1 dozen

1	2	3
20.	Reagent bottles, wide mouth, with dust-proof stoppers 100 ml	2 dozen
21	Reagent bottles, narrow mouth, with dust-proof stoppers 1000 ml	6
22	Reagent bottles, narrow mouth, with dust-proof stoppers 500 ml	2 dozen
23.	Drop bottle 100 ml	12
24	Pipette graduated 10 ml marked in 0.1 ml division	12
25.	Pipette graduated 10 ml marked in 5 ml division	12
26.	Pipette graduated 1 ml marked in 0 1 ml division	12
27	Pipette graduated 0.1 ml	50
28.	Jars with overlapping lids to store sterilized glassware 10" dia and 12" high	12
29.	Specimen jars with lids	12
30.	Coupling staining jars	12
31.	Microscopic slides (Gold seal)	6 gross
32.	Cover-slip (square) Blue star	6 oz
33.	Haemocytometer complete	2 sets
34.	Insemination catheter for cows, glass with blunt tip	6 dozen
35.	Insemination catheter for heifers—blunt and pointed	4 dozen
36.	Moulded rubber connection (rubber adapters)	6 dozen
37.	Aseptic metal containers for carrying inseminating catheters	2
38.	Rubber bulbs for suction	4
39	Syringe glass with ceramic piston 2 ml	24
40.	Semen preservation straws (in different colour)	6 gross
41.	Inseminating gun sheaths	2 gross
42.	Measuring flasks 10 ml	6
43.	Measuring flasks 100 ml	6
44	Measuring flasks 500 ml	6
45.	Funnel all glass 2" diameter	6
46.	Funnel all glass 4" diameter	6
47.	All glass automatic distillation apparatus (Pyrex)	1
48.	Aspirator bottles with syphon (10 litres) all glass	1
49.	Petri dishes 2" and 3" diameter	6 each
B. Electrical Equipment		
50.	Hot plate 7" diameter	1
51.	Hot water kettle 1.7 litre capacity (stainless steel)	1
52.	Warm cupboard for drying A.V. or hair dryer	1
53.	Hot air oven 24"x 24" x 24" double walled inside chamber made up of stainless steel-glass wool insulated, thermostatic control	1

1	2	3
54	Instrument sterilizer 24"x12"x10" or any other size	1
55.	Stove single burner	1
56	Refrigerator 165 cc	1
57.	Hot water geyser 10 litre	1
	C. Protecting Clothing and Linen	
58.	Plastic aprons	2
59.	Gumboots	2 pairs
60	Cotton aprons	6
61.	Towels	6
62	Napkins	12
63.	Linen bags to store AVs in aseptic condition	12
64.	Gloves with sleeve	4
65	Gloves latex	2 dozen
66.	Disposable plastic gloves	2 gross
	D. Semen Transport and Veterinary Equipment	
67.	Thermos flask 1.7 litre capacity	12
68	Wooden boxes with rubber lining to accommodate aluminium containers or thermos flasks	16
69.	Bicycle	1
70	Scissors curved on flat 10"	2
71	Dissecting forceps size 5"	2
72.	Wound syringe metal 4 oz	2
73.	Vaginal spaculum 4" long	2
74	Vaginal spaculum 10" long	2
75	Vaginal spaculum 8" long	1
76	Headlight with focussing arrangement	1
77	Nail clipper	1
78.	Flutter valve apparatus	1
79	Talcum powder tin	1
80.	Veterinary hypodermic syringe (Arthro) 20 ml	1
81.	Veterinary hypodermic syringe (Arthro) 10 ml	1
82.	Spare barrels for the 20 ml syringes	2
83	Spare barrels for the 10 ml syringes	2
84	Hypodermic needles 3" long	6
85	Hypodermic needles 2" long	6
86.	Swab holders (18" long for the use with bulbs A V S.)	2
87.	Breeders thermometer metal clad 110°C	1
88.	Eosin	25 g
89.	Mercurochrome	10 g

1	2	3
90.	Opal blue	25 g
91.	Nigrosine (Water soluble)	25 g
92.	Indian Ink	6 bottles
93	Carbol fuchin (B D.H.)	25 ml
94	Eosin yellow (water soluble)	25 g
95	Enamel jars with lids 10x12"	6
96.	Irrigator can E L	6
97.	Enamel trays 20"x14"x13"	2
98	Enamel trays 10"x8"x2"	2
99.	Enamel trays 8"x5"x1"	2
100.	Enamel trays with lids 10"x8"x2"	2
101.	Enamel trays with lids 8"x5"x1"	2
E. Breeding Bulls Equipment		
102	Buckets, enamel 1 with lids E.L.	2
103.	Buckets, stainless steel	2
104	Feed troughs	10
105.	Buckets G.I	6
106	Neck chains	12
107.	Bull leaders	12
108	Bull nose rings (big size)	12
109.	Bull holders	2
110.	Cotton rope 3/4"	20 kg
F. Chemicals		
111.	Vaseline for lubrication of AV	7 kg
112	Sodium citrate (A R. quality)	1 kg
113.	Absolute alcohol	5 kg
114	Iodine	200 g
115	Rectified spirit	20 litres
116.	Potassium iodide	200 g
117.	Formalin	1 litre
118.	Liquid paraffin	1 litre
119.	Immersion oil for microscope	100 ml
120	Xylol	10 litres
121.	Vim to clean refrigerators	6 tins
122.	Washing soda	10 kg
123.	Talcum powder	5 kg
124.	Cotton non-absorbent	10 kg
125.	Cotton absorbent	10 kg

1	2	3
126	Bromothymol blue 100 ml (S.D.)	20 vials
127.	Penicillin 4 lacs vials	30 vials
128	Dihdro-streptomycin sulphate 0.5 g	30 vials
G. Miscellaneous equipment		
129	Wooden stools for AVs	2
130.	Stainless steel funnels for filling AVs	2
131.	Plastic funnels	2
132	Stainless steel or aluminium tube racks	2
133.	Tripod stands	2
134.	Pipette stand	2
135	Test-tube stand (wooden)	3
136.	Wire-gauge with asbestos centre 6"x6"	4
137	Ritort stand	2
138	Filter paper 11 cm dia.	12 packets
139	Balance to weigh 100g with wt. box	1
140.	B D H capillary for pH indicator	1
141.	Research binocular microscope with built-in illuminator	1
142.	Plastic cover for microscope	1
143	Warm stage (biotherm with transformer)	1
144.	Rubber teats for pipette	4 dozen
145.	Air blower	2
146	Test-tube holders	1
147	Slide box for 1000 slides with wooden grooves	1
148.	Stand for the above	1
149.	Brushes with handle to clean bucket	2
150.	Brushes to clean variety of items	6 each
151	Glass marking pencils	2
152	Wire baskets 6"x6"x8"	6
H. Furniture		
153	Work bench 3' high	1
154.	Work bench 2' high	1
155.	Stools, all metal	2
156	Aseptic surgical instrument cabinet	2
157	Steel cupboard with glass front	1
158	Steel cupboard without glass front	1
159	Waste dressing receptacles	2
160	Wooden stand for drying linens and cones	1
161.	Washboard with sink	1
162	Microscope table	

In an artificial insemination laboratory where facilities for freezing the semen need to be provided, the following additional equipment is required:

S. No	*Name and details of articles*	*No. or quantity required*	*Remarks*
1	2	3	4
Non-consumable articles			
1.	Liquid nitrogen plant small type-capacity 2000—2500 litres a month	1	
2	Generator	1	Required as standby only in places where a steady supply of electricity is not available.
3.	Water cooling system for cooled water circulation for the LN_2 plant	1	Required only if natural water is warmer than 20^0C
4.	Water bath to keep fresh semen for initial evaluation 20"x20"x6"	1	
5.	Photo-electric colorimeter	1	
6.	Refrigerator	1	
7.	Cold handling unit	1	If resources permit, a cold room can be constructed instead.
8.	Straw clips (to hold 15 straws at a time)	100	
9.	Aspiration pump (vacuum pump)	1	
10.	Filling comb to hold 15 straws at a time	10	
11.	Wide mouth LN_2 container for freezing	1	
12	Precision balance	1	Preferably single pan
13.	Printing machine semi-automatic for printing of straw	1	Can print about 1500—2000—straws per hour

1	2	3	4
14	Drying cabinet for printed straws	1	Preferably with fan
15.	Straw distributor on ramp	1	
16	Freezing racks	30	
17	Valsallum forceps—18—20" long	6	
18.	Freezing grill	1	
Consumable articles			
1.	Straws	5 Lakh in different colours	
2.	Polyvenyle alcohol	3 kg	

Semen Bank (Storage capacity about 11-12 lakhs doses)

1.	Narrow mouthed storage LN_2 refrigerator, capacity 45000—50000 medium straws	2
2	Canister equipment for the above	2 sets
3	Narrow-mouthed medium storage refrigerators	10
4.	LN_2 containers (about 25 litre capacity)	20
5.	Goblets, large size	2000
6.	Small type refrigerators—capacity about 1000 straws	10

Field Insemination Units (300 units with maximum 4 lakh inseminations)

S. No.	*Name and details of articles*	*No or quantity required*	*Remarks*
1.	Small type LN_2 refrigerator capacity 7—10 litres	320	One for each unit and reserve
2.	A I. gun—1/2 ml or 1/4 ml	320	Twenty reserve
3.	Goblets—small size	3000	
4.	A.I sheath (for 1/2 ml or 1/4 ml straws)	4.5 lakh	

Preparation of Heat Expectancy Charts:

To detect the maximum number of animals in estrus and thereby to improve the breeding efficiency of the animals, a heat expectancy chart is a pre-requisite for any organized enterprise having animals and AI stations. It has the advantage of helping in anticipating females in silent heat. Accurate and authentic heat records of the animals are required for the preparation of the chart.

CHAPTER TWENTY TWO

Infertility in Dairy Animals

Reproductive disorders in domestic animals cause great economical losses. The major problems of reproduction in dairy animals include anestrus, repeat breeding, embryonal or fetal mortality and calf mortality. In a survey of 20,000 adult bovines, the incidence of infertility was as follows.

Heifers	22.23%
Cows	11.35%
Buffalo heifers	10.28%
Buffalo cows	6.25%
Bulls	11.65%
Buffalo bulls	3.65%

The incidence of reproductive disorders was as follows in another survey:

Anestrus	52.4%
Cystic ovaries	1.01%
Pyometra	0.91%
Metritis	8.90%
Repeat breeding	12.0%
Miscellaneous causes	1.09%

A well managed dairy herd should have 65-70 percent of cows/buffaloes conceiving on first service with an average of 1.3 to 1.7 services per conception. There should be less than 10 percent of cows with reproductive problems at any time. The calving interval from one calving to the next should be between 12 and 14 months

Lack of integration in any phase of the hormonal sequence will result in reproductive failure. This can occur mainly at the following stages of the reproductive process

—Structural and functional development of the sexual organs
—Prefertilization
—Pre-implantation
—Post implantation
—Pre-natal, neonatal and postnatal

Reproductive failure may occur as a result of abnormality in estrus, ovulation, egg reception, fertilization, egg transport, blastocyst development, implantation, embryo survival, placental development, fetal development, parturition and lactation.

The main problems of reproduction in dairy cattle and buffaloes are:

—Late maturity
—Long interval between calving and post partum conception
—Repeat breeding
—Summer anestrus in buffaloes
—Low sex libido and aberrations, spermatogenesis

Genetic, anatomic, environmental, nutritional, metabolic, immunologic, pathologic and managemental factors may contribute to the following failures:

Delayed Puberty and Maturity:

The female reproductive tract and the ovary slowly increase in size and show no functional activity before puberty. But at puberty the first estrus and ovulation are accompanied by a sudden increase in the size and weight of the reproductive system. This involves the sudden release of gonadotrophins from the anterior pituitary and gonadal hormones.

Puberty, except in seasonally breeding animals, usually occurs as the mature weight is approached and the growth is completed The age and weight at puberty varies widely between species (*Table-23.1*). Besides the hormonal and other factors, the following factors are also responsible for delayed puberty:

(i) *Season:*

Season of birth has a highly significant effect on the age of puberty. Eve lambs may be old and heavy but they do not exhibit estrus before the breeding season and will come in heat only in the breeding season (Seasonal breeding)

(ii) *Temperature:*

Experiments on cow-heifers have shown that they reach puberty at an average age of 398 days when reared at 80° F as compared to 300 days at 50° F. Moreover, the age at puberty was only 320 days. ers kept in an open shed, exposed to outside conditions

(iii) *Breeding:*

Inbreeding delays the age of puberty whereas crossbreeding hastens it to a younger age

(iv) *Sex:*

Females of all species reach puberty at an earlier age than males.

Exercise, nature of work and association are also some factors responsible for delaying puberty and maturity.

Ovarian disorders:

About 65 percent of the reproductive disorders are of ovarian origin and they may fail in the following categories:

(i) *Anestrus*

Anestrus condition in dairy animals may be physiological, nutritional, pathological and genetic in origin. Anestrus due to aging, seasonal anestrus in buffaloes, goats and sheep and anestrus due to lactation is of physiological origin Anestrus due to gonadless, ovarian hypoplasia, smooth ovary condition is genetic in nature. Anestrus may also be due to pregnancy, persistent corpus luteum, uterine pathology, embryonic death and debility.

(ii) *Atypical estrus.*

These are aberrational in exhibition of estrus by the animals

Table 23.1 The Reproductive cycle of Domestic Animals

Animal	Onset of Puberty (month)	Wt. at puberty (kg)	Av age at 1st service (month)	Length of estrus cyche (days)	Duration of estrùs hours	Time of ovula-tion hours	Optimum time for service hours	Site for deposit-ing semen into rep-roductive fact	Ovum transport time	Post partum breed-ing time (days)
Cow	8-12	200-300	14-22	18-24 (21)	12-28 (18)	10-15 hrs after the estrus	Mid to end of estrus	Uterus	3-4	60
Ewe	8-12	25-35	12-18	15-24 (16 5)	30-60 (30-35)	12-24 hrs before end of estrums	18-24 hrs After the onset of estrums	—do—	3-4	Usually the follow-ing fall
Goat	8-12	25-35	12-18-15-24	30-60 (20)	Last (26-48)	24-36 hrs day of estrums	—do— after the onset of estrums	3-4	—do—	
Mare	12-24	Depends upon mature size	2-3 (years)	19-23 (21)	4 5-7.5 (5.5)	1-2 days before the end of estrums	24 days before the end estrums	—do—	4	25-36

N B Heifers should be bred according to size and weight rather than age

(a) *Anovulatory estrus:* This type of syndrome is more common in buffaloes than in cows and is observed in animals kept at a high plane of nutrition, specially on a fattydiet. Subnormal activity of the thyroid gland and higher output of prolactin, presence of tumours and fibrosis of the ovaries are responsible for this malady

(b) *Short, prolonged and split estrus.* The initial period of sexual receptivity is interrupted by a period of non-receptivity which is then followed by another period of heat. Heifers have short estrous cycle length (19 days) while pluriparous cows have 21-22 days duration. Any deviation from 18-24 days may be taken as an abnormal estrous cycle though it may be physiological in origin

(c) *Weak or pronounced estrus* Heat symptoms exhibited by buffaloes are of low intensity compared to those of cows. Silent heat is a problem in buffalo breeding specially when AI is practised.

The interval from parturition to first observed estrus varies from 30 to 76 days in dairy cattle In general the interval from calving to first estrus is greater in cows with higher production, in cows nursing calves or being milked four times a day, in cows on a poor or low level of nutrition intake and in older pluriparous cows with 4 or more parturitions

(d) *Delayed post partum estrus* In dairy cows it may be due to delayed involution of the uterus, dystokia, retained placenta, twinning and metritis.

The cows bred before 50 to 60 days post partum have a lower conception rate (20 8 to 48.7 percent) on first service, than the cows inseminated between 61-141 days (52.5 to 57.8 percent).

(e) *Gestational estrus:* About 3 to 7 percent of the pregnant animals show estrus mainly during the first trimester of pregnancy. This may be taken as a physiological condition and the owner should ensure that the animal is not served during this period.

Cystic Ovaries:

Cystic ovarian condition in dairy cattle is becoming one of the most common causes of infertility. Cystic ovaries in cattle are characterized by follicular or luteal cysts. Follicular and luteal cysts are anovulatory cysts while the cystic corpus luteum is an ovulatory cyst.

The basic cause for the follicular cystic conditions is a failure of the hypophysis to release sufficient amounts of LH to produce ovulation and proper development of corpus luteum

Cystic ovaries affect cows of all ages from puberty to senility but are most commonly observed following the second, third and the fifth parturition. Holstein and Guernsey among exotic and Rathi and Sahiwal among Indian breeds, are the most affected. The occurence is higher in winter and in stabled and closely confined animals and is

closely associated with the level of milk production. Increased feeding, especially of rations high in protein, stimulates lactation and development of cystic ovaries. Hereditary predisposition is also responsible for cystic ovaries. Swedish Red and White cattle are more susceptible. Ovarian tumours, paraovarian cysts, ovaritis and adhesions of the ovaries either to the ovarian bursa or to broad ligaments are also some of the conditions which contribute towards infertility or sterility in cows and buffaloes

Disorders of Fertilization:

Fertilization failure in dairy animals may be due to the following reasons:

—Untimely insemination
—Insemination with aged, abnormal or less than optimum number of spermatozoa
—Aged and abnormal genitalia
—Hereditary defects and pathological conditions of the oviduct

Hereditary or Congenital Anatomic Defects:

Genes express their full character when they are in homozygous state, otherwise most of the inherited defects are caused by the autosomal recessive gene with incomplete or complete pairing. This is very dangerous because the sublethal characters are not expressed at the time of birth but in the pubertal life of the animal.

Common Hereditary or Congenital Defects:

Hypoplasia of the ovaries, agonadal condition, persistent hymen, inguinal and scrotal hernia, blind fallopian tube, blind uterine cornua, double external os, free martins, white heifer disease, abnormal shape and position of cervix, narrow cervical canal, adhesions of infundibulum to the ovary or uterine horns, presence of external or internal cyst; lack of endometrial glands, aplasia or hypoplasia of vulva.

Some Pathological Conditions of the Reproductive Tract in Dairy Animals Causing Repeat Breeding Problems:

Disease of the oviduct causes fertilization failure and embryonic mortality while disease of the uterine horns and uterus may even lead to failure of implantation, fetal death and abortions.

The etiological factors causing lesions are varied. Many lesions are secondary to an ascending infection from the uterus following abortions, retained placenta, septic metritis and pyometra. Some of the most common found in dairy animals are as follows:—

1 *Oviduct:* They may be uni or bilateral —hydrosalpinx, pneumosalpinx, pyosalpinx.

2. *Uterus:* Hydrometra, perimetritis and para metritis, endometritis, myometritis, pyometra, abscess of the uterine wall, and tumours of the uterus.

3. Cervix. (i) Cervicitis, (ii) Cysts of the cervix.

4. *Vagina and Vulva:* Vaginitis, cysts of vagina, vulvitis, and tumours of the vulva.

Disorders of Pregnancy:
These may cause prenatal mortality, spontaneous abortions, metabolic disorders of late pregnancy, parturition such as hypocalcaemia, pregnancy-toxaemia, ketosis prepartum paralysis; prolonged gestation, fetal mummification and death of the fetus.

Disorders of Parturition:
These may arise due to fetal and maternal dystokia, asphyxia of the offspring at parturition retained placenta, prolapse of vagina, cervix and uterus and trauma of the reproductive tract.

Neonatal Mortality:
The neonatal mortality up to one month age of the calf may result because of thermoregulatory failure, poor maternal nutrition, weakness of the mother and calf, bacterial infections through the umbilical cord of the young, poor maternal behaviour and delayed onset of lactation.

Immunologic Infertility:
May occur due to the antigenic nature of the semen, egg yolk used in semen dilutor, frequent and more number of inseminations done to the same animal. This may lead to repeat breeding problems

Reproductive Problems of the Male:
Infertility or sterility is as common in the male as in the female. The degree of fertility in males may vary greatly but is more easily evaluated because of the large number of females bred by each male, especially by these males used in artificial insemination. All bulls do not have the same fertility rate. High fertility and low fertility males are recognized even if the semen picture may not be significantly different. The sex drive of the male has also been significantly related to fertility. Animals with a poor sex drive should not be used for AI

The forms of infertility in males may be put in the following categories:

—Reduced or complete lack of sexual desire and inability to copulate (*Im potentia couendi*)

—Inability or reduced ability to fertilize due to pathology of the testis and accessory glands (*Impotentia generandi*).

—Miscellaneous defects of the reproductive organs-imperfect descent of testis and testicular neoplasia.

A number of external and internal stimuli can affect the reproductive behaviour of male animals. The external factors may not be overlooked while certifying the soundness of a bull for breeding purpose

External Influences:

—Auditory communication is con-

nected with sexual stimulation, the absence of which may lead to subfertility.

—Visual stimuli

—Smell from the animal showing estrus

—Photoperiod: Change of ratio of light to darkness during the day is one of the external factors affecting reproductive behaviour.

—Place of collection of semen and place of service.

—Time of collection of semen.

Before purchase each bull should be examined thoroughly for its reproductive health, specially for gonadal hypoplasia, aplasia, seminal vesiculitis and orchitis. Some bulls apparently having a good sexual desire, may exhibit a number of abnormalities in their semen. The bulls should also be free from venereal infections such as vibriosis and trichomoniasis including brucella infections.

CHAPTER TWENTY THREE

Managemental Factors Affecting Fertility in Dairy Animals

Artificial insemination, a specialized technique in animal husbandry programmes has been playing a vital role for nearly four decades. This method of rapid dissemination of improved germ plasm is now known to almost all the urban livestock owners and to most of the rural cattle breeders. Its application has further increased with the introduction of frozen semen technology

Greater success through this sophisticated tool is a sure method for increased milk production. Success depends on the vitality and quality of semen at the time of collection, subsequent methodology applied in its preservation and its proper deposition in the female genital tract. Though it is true that 'processing of the sperm cell starts with the bull and ends with the cow' the path between these two in the artificial breeding programme is hazardous, as the spermatozoa undergoes a series of laboratory processing affecting viability and fertility.

Supplying of chilled semen by many semen collection centres was considered a achievement in the AI programme a decade ago. The switchover from semen collection centres to semen banks supplying frozen semen of proven sires is a major step forward in our modern animal breeding programmes. The progress made in the last decade has been quite amazing. However, the bottlenecks in the speedier execution and popularization of this modern tool still have to be removed. The important limiting factor is the low conception rate under field conditions. The following factors contribute in influencing conception rate (1) Availability of fertile sires (2) Establishment of semen banks (3) Proper handling of frozen semen (4) Trained inseminators (5) Motivation of farmer (6) Proper follow-up programmes.

The above factors point to the need for thorough understanding of bull management, efficient handling of semen, both in the laboratory and in the field, besides cooperation from the farmer and his cow and above all, proper follow up of the artificial insemination programme.

1. Availability of Fertile Sires:

The main role of a bull in breeding is to produce good quality semen through which it transmits its qualities to its progeny. The sire is able to express its genetic

potential to the maximum through proper feeding, good exercise, protection from disease and over all good general management.

It is generally accepted that good feeding of the pedigree bull is necessary for maintaining good health and normal spermatogenesis. Hence, the bull should receive a balanced feed to be in good breeding condition. Both over and under feeding are undesirable. A fat bull is unable to mount and ejaculate in the normal way, thus limiting its breeding value. Further,, a bull deprived of vitamin 'A' shows impaired sexual behaviour. Rations containing low protein adversely affect semen volume, sperm motility and sperm concentration and libido.

Environment and diseases play vital roles in determining semen quality and subsequent conception rate. The quality and quantity of semen of farm animals is also influenced by climatic fluctuation, affecting fertilizing efficiency All the semen attributes are superior in the spring season Of all the various climatic components, high atmospheric temperature affects the quality of semen the most.

The AI programmes pose several problems such as infections from *Brucella abortus, Vibrio foetus, Trichomonas foetus,* etc which could be transmitted by the carrier bull Further freezing does not destroy all microbes and a refrigerant acts as a disease preservative The infected frozen semen is likely to spread the disease at many places, over several years Elimination of infected bulls can certainly improve the conception rate.

2. Establishment of Semen Bank:

Besides keeping the bull healthy and free from diseases, the responsibility of workers in a semen bank is heavy. Right from the collection of semen under aseptic conditions till the final storage of the frozen semen, the laboratory worker has to be alert.

There is a definite correlation between semen evaluation and subsequent fertility results.

Once the importance of laboratory evaluation of the semen for freezing, including identification of glycerol containing fraction for each bull is thoroughly understood, the working of the semen bank should not be much different from any processing plant However it should be the endeavour of the semen bank worker to maintain high precision in the semen processing task.

3. Proper Frozen Semen Handling:

Since frozen semen has greater advantages the responsibility of handling it for better results in fertility is also greater. Wherever frozen semen is transferred from the semen bank to the various field centres, it is necessary to ensure that the level of liquid nitrogen is properly maintained. Undue movement of containers should also be minimized. In the trans-

port of chilled semen adequate ice and minimum jerks should be the aim of the courier.

4. Trained Inseminator:

The field level worker i.e. the stockman is the ultimate user of this technology. Hence, the success or failure of the technology will depend on his sincerity and ability to use the technology. The prerequisites for an inseminator are complete insight into the reproduction phenomenon, particularly his ability to detect cows in heat and his knowledge of the technique of insemination. Under field conditions, the fertility rates obtained vary among different inseminators. This is due to keenness in detecting estrus properly and accuracy in depositing semen in the female genital tract.

5. Motivation of Farmer:

AI is known to most of the livestock breeders, though it has not yet acquired wide popularity and acceptance among farmers except a few progressive cattle owners. Therefore, animal husbandry workers should make an attempt to make AI a popular tool of the livestock improvement programme. Educating farmers about signs of heat, reproductive cycle in cows and buffaloes, and the actual time the cow is due to be presented for insemination, will have a positive impact on the AI programme Friendly relations between the village level animal husbandry worker and the farmer will also help greatly. Prompt assistance by the worker in the farmer's problems will earn faith in AI activities. In addition to individual contacts with the farmers, group discussions, home visits, reasoning with village elders, including demonstrating the AI working to school-going children, will evoke favourable responses to this programme.

The experience gained by scientists in operational research projects as well as in Krishi Vigyan Kendra villages is worth recording. The scientists pay regular visits to these villages, discuss problems, invite farmers for short training where all these aspects of livestock production are demonstrated. They also conduct one-day training programmes in the villages where farmers get the opportunity to solve their problems right at their doorstep. The aim of these programmes is to successfully implement the artificial insemination technique under rural conditions.

6. Efficient follow-up programme:

It is necessary to have a good follow-up of inseminated cases in the field to know the results of insemination. Where this is not done, it will not be possible to judge or evaluate the bull, the cow, the inseminator and the over all success of the AI programme

CHAPTER TWENTY FOUR

Diseases Associated with Reproduction

Abortion in dairy animals occurs as a primary sign or as a result of many infectious diseases. A number of bacteria, viruses, fungi and parasites have been isolated from fetuses and the genital tract of aborting cows and buffaloes. *Salmonella dublin* may produce enteritis and septicaemia, often abortion follows and the agent may be isolated from the fetus. *Aspergillus fumigatus,* species of genus *Absidia, Mucor* and *Rhizopus* have also been isolated from aborted fetuses. The important causes of abortion in dairy animals are listed below:

Brucellosis, leptospirosis, listeriosis, paratyphoid infection, pseudorabies, toxoplasmosis, trichomoniasis, vibriosis, infectious bovine rhinotracheitis, epizootic bovine abortion and viral diarrhoea-mucosal disease complex In addition, abortions are sometimes caused as a result of blood protozoan infections, general debility and nutritional deficiencies.

Of all the causes of abortion in cattle and buffaloe, the important infections encountered in a dairy herd are of three types, namely.

—Brucellosis,
—Vibriosis,
—Trichomoniasis

1. Brucellosis:

Brucellosis which is also known as bang's disease, is a specific contagious disease of cows and buffaloes, characterized by a chronic inflamation of the uterus and premature expulsion of the fetus, usually in the sixth month of pregnancy and later, caused by *Brucella abortus* bacterium.

Economic importance:

—Loss of calf.
—Loss of milk.
—Increased inter-calving period.
—Heavy culling in cows and bulls
—Human health hazard.

Transmission of disease.

—Ingestion of the organism either from aborted fetus, fetal membranes, uterine discharge or contaminated feed and water.
—Infected bulls who shed organisms in the semen and thus infect cows.
—Broken and injured skin.
—Infection of conjunctival sac.
—Pathogenesis following infection The organisms are established in the regional lymph glands, where they multiply for a certain time and are then carried, by way of lymphatics, to the blood and,

thus, all over the body. Infection of the gravid uterus rarely occurs before the sixth month of pregnancy. The reproductive tract is reinvaded during subsequent pregnancies by organisms located in the lymph nodes of the udder. Fetal membranes are invaded causing thickened leather-like areas Oedema and swelling of the udder and fetal membranes result in the death of the fetus and its subsequent expulsion.

Symptoms:

Abortion is most common during the third trimester of pregnancy. Usually abortion occurs only once; subsequent calvings and lactations may be quite normal. In many cases the calf may be carried to the full term but may be dead, weak or diseased at birth. The placenta is usually streaked with a yellowish lime and the cotyledons become flaccid, thick, oedematous, leather-like and covered with creamy-yellow discharge. The surfaces are necrotic. The uterus may, for a few weeks, discharge a reddish-brown vaginal fluid.

Diagnosis.

—History of late abortions in the herd.
—Clinical manifestations.
—Isolations and identification of the bacteria:
  —Aborted fetus—direct culture from stomach and lungs.
  —Placenta direct smears from chorion.
  —Uterine exudate—guinea-pig inoculation of lochia.
  —Inoculation of milk into guinea-pig and cultural examination.
—Serological tests:
  —Tube and plate agglutination test-serum, whole blood, whey, milk, vaginal/cervical mucus, semen plasma.
  —Milk ring test.

Differential Diagnosis.

Please refer to Table 24.1

Treatment:

Usually not adopted.

Control:

The control of the disease can be undertaken by calfhood vaccination programme using *Br. abortus* strain 19 vaccine. Infected animals should be segregated and positive reactors should be culled.

2. Vibriosis:

This is characterized by infertility, early embryonic death and, sometimes abortions. The disease is caused by *Vibrio fetus* bacterium and is transmitted by coitus or by artificial insemination. The disease is considered truly venereal and as such control programmes can be based on this assumption. It can be spread by contaminated instruments. Cows later develop resistance to the disease but rein-

fection is possible.

Symptoms:
The primary effect of the disease is temporary infertility. Abortions are of secondary significance. The estrous cycle becomes irregular and long. Conception takes place but is interrupted by infection. The fetus may be absorbed and a new cycle, thus, begins. When the fetus is expelled, it is so small that the abortion goes unnoticed. The variable degree of endometritis, vaginitis and cervicitis which normally follows this infection may also go unrecognized. Abortions occur a all stages of pregnancy, but mainly etween the fifth and sixth months. The placenta may be retained. The cotyledons are greyish in colour and may be covered with a caseous exudate.

The inter-cotyledonary area is red, thickened and sometimes leathery. Such placental and fetal lesions, however, also occur in brucellosis.

Diagnosis:
Diagnosis can be made by the history and clinical signs of the animal, by the vaginal mucous agglutination test and by the isolation of *V. fetus* from the female genital tract/semen and preputial cavity of the infected bull, and by the fluorescent antibody test.

Treatment and control:
The practical method of control of this disease is to use artificial insemination. The semen used should be from a known healthy bull and should be diluted at 1:25 and 500 i.u. of penicillin and 0.5 mg of streptomycin added to each ml of the diluted semen.

Usually, there are no gross uterine lesions and if reinfection is avoided 75 percent of the cows will recover in a short time, 24 percent will require 2 to 12 months and a few cows will carry the infection for a longer period. However, if no reinfection occurs, the herd may be considered free of the disease after 2 years.

3. Trichomoniasis:
This is a protozoan, venereal disease of contagious nature, caused by *Trichomonas fetus*, and is characterized by sterility, abortion and pyometra. The organism is found only in the genital tract of cows and bulls and is a strict parasite. The disease is transmitted through the contaminated hands of attendants, contaminated instruments and natural or artificial insemination. Like vibriosis, this disease is also self-limiting in nature but is often present in a herd for many months before it is recognized. Cows later develop resistance, the fertility rate goes up subsequently and may reach such a level that the owner does not bother further about it,

Clinical findings:
Infertility, repeat breeding and long irregular estrous cycles are the most common

clinical signs recognized. Infertility is caused by early embryonic death. If the embryo lives longer than 10 days, the interval between heat periods is increased. Sometimes, the pregnancy continues up to the third month, and then the abortion can be recognized. It is a common cause of pyometra in cattle and buffaloes resulting in the mortality and maceration of the fetus.

Diagnosis:

Diagnosis of the disease can be made from the history and clinical sings of the animal and by isolation and identification of the organism.

Treatment and control:

- Sexual rest of 90 days to the infected cows.
- Treatment of pyometra/culling.
- Artificial insemination from healthy bulls (free from infections).
- Treatment of infected bull.

4. Mastitis:

This refers to the inflammation of the udder and or teats and is usually caused by a wide variety of micro-organisms. Dairy animals, particularly crossbred cows, having higher productivity potential are highly susceptible to mastitis. It is one of the most important diseases in terms of economic loss to the farmer. If not treated early, it affects the quality of milk, causes total suppression of milk and sometimes renders the udder useless for further production. In addition, the contaminated/discoloured milk becomes unsuitable for human consumption and in rare cases, serves as a media for the spread of sore throat infectious to human beings.

Causes:

Many infective agents including bacteria, viruses and fungi have been implicated as the cause of mastitis, the important being *Streptococci, Staphylococci, Corynebacterium,* and *E. coli.* Certain factors predispose the animal to this disease. Thus, mastitis occurs more in high producing animals kept in bad, unhygienic conditions soon after calving. Pendulous udders, cracked and injured teats, faulty methods of milking, incomplete stripping, contaminated hands, sick milkers, excessive cold weather, presence of subclinical carriers in the herd and some intercurrent diseases also expose the animals to mastitis.

Symptoms:

Mastitis may begin in an animal at any stage of lactation. However, it usually occurs soon after calving, when the udder tissue is suddenly stressed due to heavy secretion of milk. Two forms of mastitis are recognized according to their severity; (1) acute form and (2) chronic form.

In the acute form of mastitis, the affected quarter is hot, painful, tense and tender. Milk secretion is suspended. It may be watery, straw coloured, blood-

tinged or having clots. The chronic form is not readily recognized. The cells that secrete milk becomes inactive and will be replaced by non-glandular fibrous or scar tissue. Small flakes or floculi may appear in the milk. The quarter gradually loses its soft, pliable nature and becomes very hard.

Diagnosis:

This presents little difficulty if careful clinical examination is carried out. In the diagnosis and control of mastitis, laboratory procedures are of great value. The following are the simple field tests for the rapid diagnosis of mastitis:

- Strip cup test
- California mastitis test (Mastaid test);
- Antibiotic sensitivity test.

Treatment:

The treatment of mastitis by using a disposable tube containing antibiotic ointment on the udder can be highly effective in removing infection from the affected quarter, if treated early. However, the yield of milk, is unlikely to return to normal level at least until the next lactation. Suitable treatment is advisable in all cases of mastitis in which there is a marked systemic reaction. Regarding the choice of drugs, use the one which is most likely to control the particular infection. When the type of infection is not determined, use a broad spectrum drug or a combination of narrow spectrum antibiotics with or without cortisones. Treat the udder for five days to maintain an effective concentration of the drug.

Control:

For control of mastitis, the following points should be considered:

- Infected animals should be milked last.
- All utensils used for milking, should be boiled and washed with any antiseptic solution. The teat and udder should always be cleaned before every milking.
- Sheds should always be kept clean and disinfected.
- Veterinary aid should be sought soon after the detection of the condition.
- The affected quarter can be dried off if it is incurable.
- For reduction of the infection in the herd, half a tube of any suitable antibiotic should be pushed into each quarter of the animal, when she goes dry.
- This should be repeated one week before the next calving.

5. Milk Fever:

This disease is also known as parturient paresis or hypocalcemia. It is an acute calcium deficiency, occuring in recently calved animals, resulting in a brief stage of excitement and tetany in the early stages and convulsions, paresis and coma in the later stages. This disease is usually seen in the first week after parturition in high yielding animals.

Causes:

The cause of the disease is a fall in blood calcium level associated with a disfunction of the parathyroid gland. Following parturition and due to sudden start of a large quantity of milk secretion, a large amount of calcium is secreted in the milk. In normal cases, this calcium is present in the animal feed. If, the feed lacks calcium the parathyroid gland comes into action and withdraws the necessary amount of calcium from the bones. However, in cases where this gland gets exhausted, deficiency of calcium arises and the disease appears.

Symptoms:

In the initial stage, the animal is hypersensitive, restless and gets excited. This continues for a few hours and that follows a period of paresis. In this period, the animal becomes calm, is unable to stand, sits down on the ground in such a way as if it is looking towards its flank. The animal is completely unaware of its surroundings. This condition of coma increases with time and ultimately the animal may die, if not treated by a veterinary doctor.

Treatment:

Calcium boro-gluconate or mifex-300 to 400 ml i/v for 4 days.

Prevention:

(i) Ostocalcium-B-12 Syrup, 100 ml orally for 10 days.

(ii) Mineral mixture minimix/milk min at the rate of 40-50 g per day for one month.

CHAPTER TWENTY FIVE

Artificial Breeding Records

A successful scientific artificial insemination programme must have efficient and informative record keeping. Records are essential to know about the performance of a bull, preservatibility of its semen, to make decisions regarding the number of doses and method of processing, storage and location of the semen within containers and distribution of semen. These are also essential for knowing about the reproductive performance of a cow, fertility of a bull and the efficiency of the workers involved in A.I. work.

Records maintained in the artificial breeding programme may be classified into two groups: (1) records dealing with semen collection, processing and preservation (2) records dealing with artificial insemination services. The records, can be maintained on sheets to be placed in a cabinet or in bound registers. However, it is essential to enter the data in bound registers after the same has been recorded on loose sheets.

The following records are maintained in an AI Laboratory, schedules of which are also given below:

Semen Records:

i Semen evaluation record
ii Semen processing record
iii Semen storage record
iv Despatch of semen record
v Frozen semen stock register
vi Liquid nitrogen record

A.I. Records:

i Service register
ii Daily A.I. register
iii Breedwise A.I. register
iv Bullwise A.I. register
v Heat expectancy register
vi A.I. records maintained at the village centres

Semen Records

i Semen evaluation record

Bull No.	*Semen Colour*	*Semen quantity, ml*	*Equilibration time*	*Sperm Concentration*	*Motility before freezing*	*Total number of sperms*
1	2	3	4	5	6	7

ii. Semen processing record

Bull No	*Live %*	*Sperm concentration*	*No. of sperms per dose*	*Dilution rate*	*Total number of straws*	*Half dilution*	*Total number of sperms*
1	2	3	4	5	6	7	8

Equilibration time	*Motility before freezing*	*Motility after freezing*			*Colour of straw*	*Colour of PIA powder*
		0 hr	*24 hrs.*	*7 days*		
9	10	11			12	13

iii. Semen storage record

Date	*Colour of straw*	*Colour of powder*	*Container Number*	*Canister Number*	*Goblet Number*	*Layer*	*Date of disposal*	*If shifted date and location*
1	2	3	4	5	6	7	8	9

iv. Despatch of semen record

Date	*Name and address*	*Quantity sold*	*Rate per straw*	*Total amount*	*Bull Number*	*Number of doses*	*Date of freezing*
1	2	3	4	5	6	7	8

Motility observed by lab. tech.	*Semen motility observed by the purchaser*	*Signature of the party*	*Signature of the incharge*
9	10	11	12

v. Frozen semen stock register

Date	*Previous balance*	*Quantity frozen*	*Total*	*Quantity issued*	*Balance*	*Remarks*
1	2	3	4	5	6	7

vi. Liquid nitrogen record

Date	*Production purchase*	*Consumption*	*Purpose for used*	*Name of the party by whom produced*	*Balance*	*Signature*
1	2	3	4	5	6	7

A.I. Registers

i. Service register

S. No	Animal number	Date of last calving/date of birth	First estrus	First insemination	First post parturition insemination	Subsequent insemination	Insemination result
1	2	3	4	5	6	7	8

ii. Daily A.I. register

S. No.	Animal number	Semen used	Age of semen	Last estrus	Number of insemination	Inseminator
1	2	3	4	5	6	7

iii. Breedwise A.I. register

S. No.	Date of AI	Animal Number	Previous records		Bull used	Stage of estrus	Inseminator	AI result P/NP.
			Number of insemination	Last insemination				
1	2	3	4	5	6	7	8	9

iv. Bullwise AI register

S. No	*Date of AI*	*Animal Number*	*Age of semen*	*Extender*	*Number of AI on the female*	*Inseminator*	*AI result P/NP*
1	2	3	4	5	6	7	8

vi. AI Record maintained at the village centres

Date of AI	*Daily number*	*Monthly number*	*Name and address of owner*	*Breed of Animal*	*Lactation Number*	*Bull Number*	*Previous Number of Insemination*	*Time of AI*
1	2	3	4	5	6	7	8	9

Stage of heat	*Number of straw used*	*Pregnancy result P/NP*	*Date of calving*	*Sex of calf*
10	11	12	13	14

CHAPTER TWENTY SIX

Fertility and Its Evaluation

Poor fertility in animals will act as a barrier to economic exploitation. In order to define and measure fertility levels, reliable and precise criteria are required. Fertility in dairy cattle depends on a combination of factors in the cows, heifers and bulls and ultimately, the AI centres and farms. The different aspects of fertility measures are discussed below:

Sexual Maturity on Puberty

In heifers, sexual maturity starts with the first ovulatory estrus and the development of the sexual drive leading to copulation. A substantial delay in sexual maturity may mean a serious economic loss as it would mean an additional, non-lactating, unproductive period of several months. Under tropical conditions, dairy cattle generally take around 18 months to attain sexual maturity.

In bulls, sexual behaviour with clear signs of libido, as a rule, appear quite early, at about 1 year of age. Normal levels of spermatogenesis and fertility appear gradually by around 1½ years of age.

Sexual Desire

Sexual desire or libido is present most of the time in bulls, once sexual maturity is reached. In cows and heifers it is cyclic and linked to the period of estrus

The sexual desire of bulls can be measured by the "reaction time", that is, the time a bull needs from its first contact with a cow standing in estrus until complete erection and copulation have been achieved. The rapidity rather than the intensity of reaction is a reliable parameter for sexual desire. Breed, age and physical condition have a profound effect on the reaction time in bulls

Sexual desire can also be measured by the "exhaustion" test, that is, the number of services performed by the bull within a given period of time. A bull with high service capacity may copulate 10 times in a period of 7 hours, while a bull of low service capacity may copulate only 3 times. However, the degree of sexual desire is by no means directly correlated with their fertilizing capacity

In cows and heifers, however, subnormal expression and length of estrus are a serious cause of temporary infertility, since opportunities for fertilization are lost

Non-Return Rate

The non-return rate is the percentage of cows and heifers which do not return in heat within 30 days of insemination. It is clear that the non-return rate will be higher after 30 days than after 60-90 days, since it will include the late returning cows in which a late embryonic mortality has occurred, or in which an estrual period has gone unobserved. The non-return rate is a most useful parameter for the combined fertility of bulls and cows, and its reliability is increased as time elapses after breeding The non-return rate after 30 days should exceed 70 percent, while at 60-90 days, 70 percent is a very satisfactory figure.

Conception Rate

The conception rate is the percentage of females actually pregnant after the first breeding At three months after breeding, the figure will be at least 5 percent less than the non-return at 30 days The final pregnancy rate is the percentage of all cows and heifers served that became pregnant, after one or more services or inseminations. A final pregnancy rate of 80 percent is considered satisfactory. As a rule, the conception rate is determined by clinical pregnancy diagnosis, which gives quite reliable results from about 50 days after breeding.

Calving Rate

The calving rate is the percentage of cows served which calve at term and have optimal chances of producing a living calf. The figure is lower than the final conception rate because an average of 3 percent of abortions has to be allowed for.

Index of Pregnancy or Efficiency Figure (S/C)

S/C stands for "services per conception", but in the strict sense this figure is the number of services or inseminations per final pregnancy A total of 140 inseminations for 100 cows and heifers, finally yielding 80 of them as pregnant, gives an index of 1.4 per inseminated cow and one of 1 7 (S/C 1.7), per conception which can be considered as normal

Calving Interval

This interval is the period of time between two successive calvings; it is the sum of the gestation period and the calving to conception interval or "days open" period. The optimum calving interval for cows is often stated to be 12 months, but most stockmen are happy with an average interval of 13 months. A figure for calving interval is needed for an adequate evaluation of fertility. If a cow comes in heat 4 or 5 months after calving and becomes pregnant after the first insemination, she will score a very good non-return, conception-rate and an S/C figures but due to the prolonged delay postpartum, her reproductive efficiency is far too low. Several factors can influence the calving interval. For high lactating heifers, the owner may intentionally withold service at the first and second

heats postpartum, in order to give them better chances for growth, lactation and fertility. However, in the records this induces an artificial falsification of the calving interval parameter. If the stockman is accustomed to breeding all cows at first heat, even if this appears before 50 days postpartum, he may achieve his objective of a shorter calving interval, but he will undoubtedly always have a decreased non-return rate. In general, the postpartum anestrus period is markedly longer after a first calving than after the second and later calvings. This is probably due to a diversion of foodstuffs for use for skeletal growth which, added to the requirement for milk production, results in a postponement of the first ovulation and first heat. A very marked delay in the appearance of the first estrus after calving, can be caused by allowing a calf to suckle the cow.

Longevity, "Stability" or Working Life

The figure for this factor is a number of years during which bulls and cows maintain a normal reproductive capacity; the factor is particularly important in dairy cattle. Many dairy breeds fail to obtain an average of 5 calvings and lactations per cow. This corresponds to an age of 7 to 8 years from birth. Milk yield increases in successive lactations and stays at that peak level up to the seventh, eighth, or occassionally even the tenth lactation.

Individual cows may go well beyond 10 lactations while exceptionally high yielding dairy cows have completed 16 or more lactations, attaining ages of over 20 years.

Spontaneous Calving and Difficult Calving:

A spontaneous calving needs no help, while cases of dystokia, may need reposition, traction, fetotomy, or caesorotomy. There is an enormous variation in respect of ease of calving between breeds 1 to 30 percent of calvings requiring human assistance and with a markedly higher incidence or dystokia in heifers than in cows.

The economic importance of difficult calving lies in the cost of obstetrical help, disturbed general health, lower milk production, subsequent infertility of the dam, and a great increase in perinatal mortality of calves.

PART IV

LACTATION

CHAPTER TWENTY SEVEN

Animal Nutrition in Relation to Reproduction

Among the factors which predominantly limit the reproductive ability of an animal, nutrition happens to be the foremost. The dairy cow requires five major classes of nutrients viz., carbohydrates, fats, proteins, minerals and vitamins besides sufficient quantity of water. All five are essential in proper quantity and proportion for normal health and productive purposes. Next to water, the greatest requirement is for carbohydrates, followed by proteins. These are limiting factors for high milk production. Although almost all nutrients function synergestically for a specific purpose, the deficiency of one or more substances may affect the reproductive system more than other systems. All the nutrients together provide the energy requirements of the animal, though individually, they provide different contributory components to total energy.

Energy:

The simplest definition of energy is 'the ability to do work'. A dairy cow obtains its energy from the carbohydrates it consumes and it uses this energy for a variety of functions in her body A growing heifer needs extra energy for the tissues that she is adding to her body, a pergnant cow needs additional energy for building up the tissues of the fetus, and a lactating cow requires more energy to manufacture milk When feed is restricted, as in partial starvation or under feeding, the body weight is lost and reproductive efficiency is decreased which is manifested by irregular estrus and low fertility. Under feeding of heifers delays the onset of the first estrus. The level of nutrition during the growth period has a marked effect on the age at puberty. Poor feeding results in stunted growth and delayed sexual maturity. It has been experimentally proved that the expression of heat symptoms and the conception rate could be increased by increasing the intake of digestable energy from 14 0 mega calories (DCP 0.2436 kg, TDN 3 182 kg) to 20 5 mega calories (DCP 0.57 kg, TDN 4 652 kg) in cattle

Under ideal conditions, a dairy cow should produce a calf once each year. Energy requirements for the developing fetus are very small during the first 6 months of pregnancy but increase sharply during the last 3 months. Hence, provision should be made to feed extra energy in the form of a concentrate mix-

ure during the last 3 months of pregnancy. Restricted feeding at this time leads to an increased postpartum anestrus period. Further, animals fed on a high plane of nutrition have heavier calves. It has also been experimentally proved that under-fed animals experience difficulty in calving, postpartum estrus is delayed and intercalving period is increased.

In males too, a poor plane of nutrition results in delay in attaining puberty. Motile spermatozoa was found in well fed young bulls earlier as compared to bulls brought up under low energy rations. This also affects the sperm production. It is difficult to overcome such condition even after subsequent improvement in feeding. Adult bulls are less susceptible to under-feeding as compared to young ones.

Carbohydrates:

Carbohydrates, particularly cellulose (forages) and starch (concentrates and grains) make up the largest percentage of dairy cattle ration. Ruminants like dairy cattle can utilize cellulose and similar compounds as an energy source after fermentation by ruminal microorganisms Most of the carbohydrates in the diet of the animal are fermented by ruminal microorganisms into volatile fatty acids (VFA) like acetic, propionic and butyric acids. Different rations yield different proportions of these acids. Rations that contain a high proportion of forage and other roughage types favour the production of acetate in the rumen while feeding with a ration of grains or concentrate favours increased propionate production. These acids, on absorption provide energy or carbon source to the animal. Thus in ruminants, glucose metabolism is very limited.

Fats:

A disproportionate content of fat in the diet or a tendency on the part of the animal to synthesize more fat from manomers that are absorbed, results in decreased reproductive efficiency Over feeding of animals resulting in 'fattening' leads to decreased fertility as compared to animals that have less tendency to synthesize fats.

Proteins:

Protein malnutrition is common is some countries of the world including India, both in human beings and domestic animals. In order to be utilized by an animal, proteins must be broken down by digestion to amino acids of which they are made. In ruminant animals like cows, the microorganisms are capable of synthesizing proteins for their cells from amino acids and non-protein nitrogen derived from the cow's diet. These 'microbial' proteins are, subsequently digested and absorbed by the cow, giving her a source of all essential amino acids. Therefore, the quality of proteins is not of much importance. However, lack of proteins in the diet is liable to lead to infertility as a generalized effect. Extra

protein is needed by a pregnant cow for the developing fetus especially during the last 2 months of pregnancy. It is during this time that the bulk of fetal growth occurs. Adequate protein to energy ratio is essential for the normal reproductive process also. A wide protein to energy ratio is detrimental to many reproductive processes. It has been experimentally proved that inadequate feeding of proteins during late pregnancy could lead to increased postpartum estrus interval. In males, protein malnutrition results in decreased sperm concentration.

Minerals:

There are at least 15 mineral elements that are known to be required by dairy cattle. Some are required in major quantities (major or macro minerals) and others in minor quantities (micro or trace minerals) depending upon the requirement. Calcium, phosphorus, sodium, chlorine, magnesium, potassium and sulphur, are major elements (minerals), whereas iron, copper, molybdenum, manganese, zinc, cobalt, iodine and selenium are micro or trace elements (minerals). Under normal conditions, minerals which are most likely to be needed by the cow in greater amounts are provided in rations as a component mineral mixture. Some minerals are obtained through plant resources. Hence, in the areas where there is a deficiency of a particular element, that particular element needs to be incorporated into the diet. Deficiency of minerals also lead to disturbances in fertility, thus, affecting reproductive efficiency.

Major minerals

(i) *Calcium and phosphorus:* These two are the most important minerals for a dairy cow. They are the main constituents of bones and teeth. Much of the calcium content of the body is depleted through milk. Dietary requirement of calcium also increases during pregnancy, for development of the fetus. Rations low in calcium result in reduced growth and development of calves. Calcium deficiency may also lead to feeble uterine contraction and difficult parturition. Phosphorus is involved in energy metabolism and many metabolic functions of the body, besides being a major constituent of bones. It's deficiency will lead to impaired energy utilization, reduced breeding efficiency, delayed maturity, irregular estrus and anestrus conditions.

(ii) *Sodium and chlorine:* Sodium chloride or plain table salt is needed in greater amounts than that provided by most rations. Commonly it is provided for dairy animals in the form of loose or block salt. Its deficiency leads to loss of weight, and unovulatory heats.

(iii) *Magnesium and potassium:* Deficiency of magnesium occurs when animals graze on pastures in areas where there is a deficiency of magnesium in the soil. Potassium is required for normal muscle irratability. Forages which are high in potassium may interfere with

magnesium utilization and causing grass tetany.

(iv) *Sulphur:* Ruminal microorganisms can use inorganic sulphur to synthesize sulphur containing amino acids particularly when non-protein nitrogen substances like urea are fed to the cattle.

Trace minerals:

(i) *Iron:* Iron deficiency is not a problem but deficiency (less than 50 ppm) may result in anaemia and sexual depression.

(ii) *Copper:* In certain areas the soil is deficient in copper High molybdenum can also cause a condition of copper deficiency. The requirement of copper is low (5 to 8 ppm) and its deficiency will lead to suppressed ovarian function, inactive ovaries and atonic uterus. Fertility can be restored by supplementation of copper in the form of copper sulphate.

(iii)*Molybdenum:* Forage containing 20 ppm of molybdenum results in induced copper deficiency. Such situations require copper supplementation.

(iv) *Manganese* A deficiency of manganese (less than 40 ppm) results in impaired growth, skeletal abnormalities, reduced fertility and birth of abnormal calves.

(v) *Zinc:* Zinc is involved in several enzyme systems. It is required at a level of 40 ppm in rations (dry matter basis). Most common feeds do not contain that amount. Zinc, along with Vitamin A plays a vital role in maintaining fertility.

(vi) *Cobalt:* This is an essential part of Vitamin B_{12} Cobalt deficient areas occur throughout the world. Animals with cobalt deficiency have poor appetite, lose weight, become weak and anaemic. Along with copper, it is an essential element for reproduction. Its deficiency (less than 0.1 ppm) will lead to retarded sexual development

(vii) *Iodine:* Much of the small amount of iodine in the body is contained in the thyroid gland as thyroxine and diiodotyrosine, whose principle function is regulation of the metabolic function. Iodine deficient areas have been responsible for iodine deficiency which is characterized by goitre, calves that are born weak, or prematurely born dead.

(viii) *Others:* Selenium and fluorine deficiency symptoms hardly occur but their toxicity symptoms are always more pronounced.

Vitamins:

Vitamin deficiency like mineral deficiency does not usually occur under natural conditions, except when adverse circumstances prevail. Cattle can synthesize adequate amounts of Vitamin B complex and Vitamin K in rumen and Vitamin C in the body tissue. Consequently, the only vitamins required in the ration of a cow are fat soluble Vitamins A,D and E. However, all vitamins except Vitamin C are needed in the diet of a young calf until ruminal activity is sufficient to fulfil its needs.

(i) *Vitamin A:* Forage consumed by cattle contains carotene which is the precur-

sor of physiologically active Vitamin A. Under natural conditions, green feeds provide an adequate amount which is converted to Vitamin A, required essentially for multiplication of cells of germinal epithelium for the formation of ova and sperms. Cows grazing on dry range for extensive periods without access to green forage suffer from avitominosis A. A deficiency of Vitamin A causes many problems like night blindness, lack of coordination, increased susceptibility to infection, abortion, birth of dead, weak or blind calves and decreased fertility. It has been experimentally proved that cows receiving low levels of vitamin have low fertility, retained the placenta after parturition and exhibit weak sexual drive and estrus symptoms. Deficiency of Vitamin A in bulls delays sexual maturity, and results in abnormal sperms and decline in sperm motility.

(ii) *Vitamin D:* Cows exposed to sunlight or eating sun-cured forage do not need supplementation with Vitamin D. The most common symptom of deficiency of this vitamin is rickets. It also results in failure to show estrus or heat symptoms in cow.

(iii) *Vitamin E:* All green feeds are a good source of Vitamin E. Additional Vitamin E is required to prevent decreased fertility. There is a close relationship between Vitamin E, Vitamin C and cystine. The specific effects of Vitamin E are, however not clear.

Thus, it is a well established fact that nutrition plays a significant role in an animals reproductive process, which is most important event in life. A well fed cow will seldom be problematic with regard to reproduction.

CHAPTER TWENTY EIGHT

Mammary Development and Ultra-Structure of Mammary Glands

The mammary glands are distin guishing characteristics of mammals. These are modified skin glands and are present in both males and females. In the latter, they develop well during postnatal life. In cows, they are situated in the inguinal region and are connected with the abdominal cavity through the inguinal canal. Four mammary glands of the cow are grouped together into a structure called the udder. Each gland is known as the quarter of the udder and is an independent structure in itself. If we look from behind, a longitudinal groove-the inter mammary groove—separates the right and left halves of the udder. The front and rear quarters seldom show any clear line of demarcation because of the lesser amount of connective tissue. The rear quarters are usually larger than the fore quarters and possess more secretory tissue. A large udder has high milk production as it possesses more secretory capacity. The udder is covered with skin which has little supportive action but protects the udder from abrasions and may prevent excessive swaying of the gland when the cow is moving.

Associated with each gland is a teat which serves as the exit for secreted milk. The skin of the teat is smooth and free from hair. No sebaceous or sweat glands are found in it. Cows with funnel shaped teats produce more milk than cows with cylindrical teats. Extra teats (supernumerary teat) are sometimes found which may or may not have the patent streak canal. These sometimes prove dangerous for udder health, as they may lead to the passage of infection into the mammary system.

The opening through the tip of the teat (the teat or streak canal) leads into the teat cistern or cavity within the teat. At the place where the cannal opens into the teat cistern, there are a series of four to eight radiating folds in mucosal lining of the sinus known as furstenbergrosette. At its upper end, the teat cistern communicates through a circular opening with the gland cistern. The ducts coming from the alvcoli or secretory tissue join into the gland cistern.

Mammary Development:

1. *Development from birth to puberty.* At birth, the mammary gland consists of a restricted duct system and little connec-

tive tissue and fat. Thereafter, the duct increases in length and number due to further branchings. The end buds which are observed at this stage are formed of solid masses of epithelial cells and should not be mistaken for alveolar tissue. The quarters continue to grow in size, partially due to the deposition of odipose tissue until the front and rear quarters approach each other and finally become joined at the base. In addition to an increase in udder weight of cows from birth to puberty, there is an increase in the udder capacity also. In calves, a shifting growth pattern relative to body surface has been observed. The growth of mammary glands during the first few months of life is in proportion to the general body growth-isometric. Before puberty, it grows at a faster rate-allometric. After puberty, the body grows at a faster rate than the mammary gland.

2. Development from puberty to conception: After puberty, the extent and type of mammary development is influenced by the number and type of estrous cycles experienced by the species. In acyclic species like the rabbit in which ovulation is induced, marked development of the mammary duct system occurs during estrus, whereas in cyclic species the mammary glands develop during subsequent estrous cycles

3. *Development during pregnancy:* Alveoli are not formed until conception. In the early stages of pregnancy, the duct system continues to develop, followed by rapid proliferation of the lobule-alveolar system. Throughout the udder, groups of alveoli begin replacing the fatty tissue. The amount of mammary development can be judged by the size of fat pad into which it is growing.

4. *Development during lactation:* Mammary growth continues during the early days of lactation among mice, rabbits and guinea pigs. In contrast to these species, mammary growth does not occur in sheep after parturition In cattle also, there is no evidence of mammary growth in early lactation.

Internal Structure of Mammary Glands:

Suspensory apparatus: This includes skin, connective tissues, suspensory ligaments and tendons. All these help to hold the udder in proper position. The udder is separated into halves by the median suspensory ligament, which attaches it to the body of the cow.

Teat and gland: The stretified squamous epithelium which lines the streak canal provides strength besides preventing the entry of infective organisms. The teat cistern is lined with 2 to 3 layers of cells. The gland is formed by the opening of the larger ducts draining the lobes. These larger ducts upwards divide and subdivide into smaller ducts and ultimately terminate in the alveolar duct. Each alveolar duct is connected with one

alveoli. The gland cistern and the larger duct are lined with two layers of epithelium. The alveolar ducts have single layered epithelium. The alveoli are special structure and are lined by epithelial cells which rest on a basement membrane. Surrounding the alveoli is the myoepithelium, the contractile element which is sensitive to oxytocin. Each alveolus is also surrounded by a capillary network which supplies the nutrients for the synthesis of milk. Observation of the epithelial cells with the help of an electron microscope indicates that the components of these cells are comparable to those found in most cells.

CHAPTER TWENTY NINE

Factors Affecting Mammary Synthetic Activity, Synthesis of Milk and Its Endocrine Control

The onset of pregnancy results in the beginning of the formation of alveoli in the mammary gland. As the pregnancy advances, the number of alveoli also increases. Secretion of milk slowly increases during pregnancy, but it is in the immediate periparturient period that copious quantities are recorded. Indices of initiation of milk secretion are mainly the appearance of: enzymes in mammary tissue specific for secretion of milk, lactose, casein, triglycerides, citrate synthesis in the mammary tissue; marked increases in mammary RNA; differentiation of organelles in mammary epithelium; histological evidence of secretion in the alveolar lumen; and secretion of copious quantities of milk immediately preceding parturition.

During the first phase of lactogenesis, limited secretion of an extracellular type fluid occurs. The second phase occurs a few days before parturition, and consists of copious secretion of milk. Various aspects of lactogenesis are regulated by a number of hormones; however, for their action, a high concentration of progesterone, usually associated with gestation, must be reduced.

Endocrine Control of Initiation of Milk Secretion (Lactogenesis):

1. Prolactin: The initial increase in mammary enzymatic activity of many species is not associated with an alteration in serum prolactin concentration. The major surge in prolactin secretion occurs immediately before parturition, and is more likely to control the second phase of lactogenesis responsible for secretion of copious quantities of milk. In agreement with this concept, prolactin occupancy of mammary receptors is reasonably constant, during pregnancy and increases only during the second phase of lactogenesis associated with secretion of copious quantities of milk.

2. *Growth hormone:* There is little evidence that growth hormone *per se* is lactogenic; rather growth hormone synergizes with prolactin and glucocorticoids to initiate lactation. In a hypophyctomized

goat, prolactin and glucocorticoids were lactogenic, but for complete restoration of milk yields, growth hormone and triiodothyronine were required. The growth hormone in sera from several species does not change greatly as pregnancy advances until parturition when growth hormone increases markedly. Thus, it is likely that if the growth hormone affects lactogenesis, it does so primarily during the latter stages of lactation.

3. *Adreno-corticotropin [ACTH] and glucocorticoids:* The mechanism whereby glucocorticoids exert their physiological action requires binding of the hormone to receptor molecules within the mammary cell. There are a few receptor sites in mammary cytosol fractions of virgin or pregnant cows as compared with lactating animals Another aspect of glucocorticoid function is the presence of corticosteroid binding globulin (CBG) in the sera. This protein binds and inactivates glucocorticoids in the blood, and increases in advancing gestation in several mammalian species. During the periparturient period, CBG decreases and free corticosteroids increases markedly. Thus, availability of large amounts of corticosteroids occurs coincident with lactogenesis.

4. *Estrogens:* There is no evidence to suggest that estrogens definitely induce mammary cells to secrete. The prepartum changes in estrogens most likely stimulate secretion of prolactin, growth hormone and glucocorticoids, which in turn, directly affect mammary secretion.

5. *Prostaglandin F_2 Alpha:* Prostaglandin F_2 alpha being luteolytic substance decreases circulating levels of progesterone. However, exogenous administration of PGF_2 alpha also causes marked release of prolactin, growth hormone and glucocorticoids, which may play a direct role in lactogenesis.

Maintenance of Lactation (galactopoiesis):

To maintain lactation, the number of mammary cells and the synthetic activity per cell must be maintained. To accomplish this, several other hormones are essential. In general prolactin, growth hormone, ACTH (or glucocorticoids), TSH (or thyroid hormone), insulin, and parathormones are included in this category.

1. Prolactin: It has been shown that milking causes a sharp release of prolactin in to the serum. In cattle, this increase occurs 2-3 minutes after the stimulus is applied and remains elevated as long as tactile stimuli are applied to the teats. When the milking stimulus is removed, prolactin returns to base line concentrations within 35-40 minutes. Prolactin concentration is higher at night and lower during the day, in lactating cows, and again, is higher in the summer than in winter. It has been found that

decreased level of prolactin in goats and cows does not suppress milk yields. This indicates that prolactin has no effect on established lactation in ruminants.

2. Growth hormone: The circulating levels of growth hormone are not altered by milking stimulus. However, the capacity of the anterior pituitary to release growth hormone is increased with elevated milk secretion which is generally encountered in early lactation.

3. ACTH and glucocorticoids: Suckling or milking causes release of ACTH from the pituitary and this in turn, results in increased levels of circulating adrenal corticoids. Cows do not lose their ability to discharge corticoids as lactation proceeds. At low ambient temperatures, high-producing cows have a higher concentration of glucocorticoids than low producers. And at a higher ambient temperatures, glucocorticoids decrease much more in high producers than in low producers. These variations in glucocorticoids' response to ambient temperature may explain why high producing cows are less persistent than low producing cows during hot weather.

It can be concluded that glucocorticoids are essential for lactation, but either increased quantities above normal or extremely low concentrations decrease lactation.

4. *Thyroid hormones:* There is a little question that exogenous administration of thyroxine (T4) or tri-iodothyronine (T3) will markedly stimulate lactational performance in both ruminants and non-ruminants. The general conclusion to be reached is that the thyroid strongly influences milk synthesis but its secretions are not absolutely essential for the process.

5. *Parathormone:* Large quantities of calcium are secreted in milk every day. To cope with this, the parathormone increases calcium liberation from bones, increasing calcium absorption from the gut and increasing kidney calcium reabsorption.

6. *Insulin:* There is almost universal agreement that administration of insulin to lactating dairy cows decreases milk production. However, if blood glucose is maintained, insulin will not inhibit lactation suggesting that it is hypoglycemia rather than direct effect of insulin that is the cause of reduced milk yield.

Milking Stimulus and Milk Let-down Process:

Lactation cannot be maintained till the milk is removed from the mammary gland. Removal of milk is facilitated by the frequent release of oxytocin from the posterior pituitary at the time of each milking. The milking stimulus through the hypothalamus is responsible for this release. Oxytocin so released in the circulation acts on the myoepithelial cells surrounding the alveoli and brings about their contraction As a result of their con-

traction, the milk present in the alveoli and smaller ducts is forced down, which is obtained at milking. Oxytocin has a short life in the animal system and is readily destroyed by the liver and kidney. Its half-life is about 45 seconds. This signifies the importance of faster milking The time lapse between the milking stimulus applied and the complete let-down of milk is called milk "let-down" time. Milk let-down time varies from cow to cow and is further influenced by the quantity of milk present in the udder at the time of milking. On an average, cows take about 45 seconds to let their milk down, however, buffaloes take more time than cows. The release of oxytocin from the posterior pituitary during the act of milking is intermittent and is continous for 2-3 minutes.

CHAPTER THIRTY

Factors Affecting the Quality and Quantity of Milk

Milk composition and production are the results of interaction of many elements within the cow and her external environment. The quality and quantity of milk produced by dairy animals is affected by various factors i.e. genetic, physiological, managemental and environmental. Feeding is environmental but taken up under managemental factor. Some physiological causes of variation may be controlled but others cannot. As an example, diseases may be kept at minimum by good feeding and management. The environmental factors, likewise can also be controlled by regulating the month of calving, length of dry period and calving interval. Dairyman can alter many of these factors to achieve greater milk production and increased profits.

1. Genetic Factors

(i) Species differences: The quality and quantity of milk produced by dairy animals like cows, buffaloes and goats differ. Buffalo milk has more milk fat and total solids, compared to other dairy animals. There are obvious differences in milk composition and yield among the various breeds of dairy cattle. Fat is the most variable constituent of milk, whereas minerals (ash) and lactose are the least variable. Differences in gene frequencies controlling the quantity and quality of milk components largely account for the average genetic differences among breeds. Among the exotic breeds, the Holstein Friesian produces the maximum quantity of milk and the Jersey, the minimum. However, the Jersey produces the maximum quantity of milk fat. Among Zebu breeds, the Sahiwal, Red Sindhi and Tharparkar breeds are considered to be good producers. The fat percentage of indigenous breeds is higher compared to exotic breeds.

2. Physiological Factors

(i) Age: There is individual variation in milk production as the animals' age advances. Milk yields increase at a decreasing rate until about 8 years of age, depending upon the breed, and then decrease at an increasing rate. Mature cows produce about 25 percent more milk than the first calvers. Increased body weight accounts for about 5 percent of this increase, whereas the remaining 20 percent of the increase is the result of

increased development of the udder during recurring pregnancies.

(ii) Stage of lactation: Milk produced at different stages of lactation varies markedly in composition. The secretion produced by the udder immediately after parturition is known as colostrum. The composition of colostrum is considerably different from the composition of normal milk. Usually, a period of 3 to 5 days postpartum is needed for the secretion to return to the normal composition of milk. During the colostrum period, the total solids, specially the globulin fraction (protein), are elevated. Following parturition, the daily production of milk tends to increase in the cases of most cows from 15 to 30 days. Higher milk producing cows usually take a longer time than low producing cows to achieve peak production. After peak is attained, milk production gradually declines. Fat percentage in milk decrease slightly during the first 2 to 3 months of lactation and then increases as total production decreases with advancing lactation. Milk protein content gradually increases with advancing lactation while milk lactose remains unaffected.

(iii) Lactation number: The order of lactation affects the quantity of milk. Zebu animals attain peak lactation yield in the second and third lactation. However, in crossbred and exotic breeds the tendency is to attain peak lactation yield in later lactations.

(iv) Gestation: Gestation (pregnancy period) in itself does not influence the composition of milk but it may indirectly cause the cow to go dry.

(v) *Milk secretion rate:* Milk secretion rate is rapid and relatively constant for 8 to 10 hours after milking and lowest just before and during milking. However, as milk accumulates, during the intervals between milking, intramammary pressure increases and milk secretion rate per hour decreases. The capacity of the udder to hold and secret milk has a major influence on the milk secretion rate. Usually, large udders produce milk at a greater rate than smaller glands.

(vi) Variation in udder quarters: Both the amounts and quality of the milk drawn from each quarter vary. On an average, the fore quarters produce 40 percent and the rare, 60 percent of the milk.

(vii) Age at first calving: The age at first calving affects the quantity of milk. If the age is more, then the first lactation yield will also be more, but this is not a desirable trait as the lifetime production is reduced.

(viii) Estrus period: Estrus may temporarily depress milk yield. This has been attributed, among other reasons, to disturbed psychobehavioural conditions in the animal, reduced feed itake, excitement and altered endocrine profile of the animal.

(ix) Size of the animal: There is direct relationship between the milk yield, fat yield and the size of the animal. The size, however, is not the most important consideration for economical production. Though large cows produce more milk than small cows, the milk yield does not vary in direct proportion to body weight.

3. Environmental Factors

(i) Season: It has been recognized that there is a seasonal effect, upon both quality and quantity of milk, of dairy animals. The amount of milk is reduced in summer and under environmental stress conditions. The alteration in yield under such circumstances is also accompanied by change in milk composition.

(ii) Exercise: Exercise tends to increase slightly the percentage of fat in milk. Too much exercise reduces the milk production.

(iii) Excitement: Disturbances at the time of milking cause the cow to hold up the milk because of the production of the hormone adrenalin.

4. Managemental Factors

(i) Effect of feed: Underfeeding cows reduces milk production and lactose percentage but increases the fat, protein and mineral content of milk. Feeding an adequate ration reverses these symptoms. As a general rule, any ration that increases milk production usually reduces the fat percentage of milk. Of the three energy producing food nutrients (carbohydrates, fats and proteins), carbohydrates least influences milk secretion, provided sufficient calories are allowed for energy requirements. There are a number of methods of feeding that depress milk fat percentage and simultaneously stimulate higher milk production. The common practice is a restricted roughage and high grain ration. Protein supplements are relatively the most expensive feed ingredient of the dairy animal. Cotton seed cake has been reported to increase the fat in milk whereas cod liver oil tends to reduce the fat percentage. Urea and other non protein nitrogen sources like biuret are extensively being used in the conventional feed mixture. Urea, with molasses or starch, is recommended for lactating animals. Studies have also been conducted to feed urea with sodium bicarbonate. Calcium and phosphorous and other elements are required in sufficient quantities by the body for production of milk.

(ii) Intervals between milkings: The effect of the length of intervals between milkings on milk production is influenced largely by the individual characteristics of the cow, such as, udder capacity, stage of lactation and the amount of milk produced. If the milking is increased from twice to thrice in 24 hours, the quantity of milk increases by around 10 percent.

(iii) Method of drying: The various methods of drying the cow, affect the milk yield. The abrupt cessation of milking is the most desirable method but it can have some effect on udder health The cows known to have udder infection in one or more quarters should be dried off by intermittent milking.

(iv) Dry period: Cows should be given a rest period of 6 to 8 weeks between lactation Either shorter or longer dry periods will reduce subsequent milk production.

(v) Calving interval: Like the dry period, calving intervals affect the milk yield. If the calving interval is longer, the animal will have slightly more yield in the ensuing lactation but its lifetime production will be reduced

(vi) Effect of drugs and pesticides: Many drugs, including pesticides used in the treatment of cattle diseases are excreted into the milk. Such milk should be discarded to prevent the drugs from entering human food supply. Certain drugs like Strychnine and Pholoridgin, have been used to increase milk yield or the fat percentage

(vii) Diseases: Many diseases, especially mastitis, ketosis, milk fever and digestive upsets adversely affect milk production and alter the composition of milk. Mastitis usually decreases casein. Ketosis may seriously reduce the yield of milk because of the lack of blood sugar.

CHAPTER THIRTY ONE

Energetics of Milk Production

Energy is obtained by the animal through its feed (gross energy). Some amount of energy is lost in faeces (faecal energy), gases (gaseous energy), urine (unrinary energy) and by way of heat increment (energy incident to the gastric process). The net energy value of feed is derived by sub-tracting faecal, gaseous, urinary and heat increment energies from the gross energy supplied to the animal through feed. This net energy is used by the animal to maintain its basal requirements of the body and milk production.

The energetic efficiency of milk production is a biological index of "dairy merit". The designation of dairy ment is related to the economy of milk production This represents the biological efficiency of milk production as measured by the percentage of consumed total digestable nutrients (TDN) energy which is converted into milk energy.

$$\text{Dairy Merit} = \frac{\text{Milk energy production}}{\text{TDN energy consumption}}$$

Energetic efficiency is the ratio of the output energy to input energy. In dairy animals, the output energy is milk. Gross energetic efficiency (GEE) of milk production of a dairy animal is the percentage of available energy to be converted into milk energy.

$$\text{Gross Energetic Efficiency (GEE \%)} = \frac{\text{Energy output in milk} \times 100}{\text{Energy retained from feed.}}$$

In this case, the input energy is used by the animal not only for milk production but also for maintenance. The net energetic efficiency (NEE) is the energy available to the animal for only productive purposes.

$$\text{NEE} = \frac{\text{Energy output in milk}}{\text{Total energy input} - \text{Maintenance energy}}$$

Studies on the energetics of milk production among various breeds of cows, buffaloes and goats reveal that factors like breed, feed, ration combination and environmental conditions, affect the conversion efficiency. Different breeds of dairy animals have different efficiencies with which they can convert 'feed energy'

to milk energy. Further different breeds produce milk differing in its composition The calorific value of the milk varies with its composition specially with the fat percentage. Milk of different fat percentages can be converted into one common unit-milk containing 4 percent fat designated as "fat corrected milk" (FCM), which has calorific value of 750 calories/kg of milk. This conversion facilitates the comparision of milk from different sources. For example, 4 percent FCM goat milk has virtually the same calorific value as 4 percent FCM cow's milk. FCM is calculated by a formula.

$$FCM = 0.4\ M + 15\ F$$

M is the weight of milk (in kg) and F is the weight of fat (in kg). Once the FCM is calculated the energetic efficiency is calculated by

$$\text{Energetic Efficiency (\%)} = \frac{\text{FCM (kg)} \times 750^{*}}{\text{TDN (kg)} \times 4000^{\dagger}} \times 100$$

† Calorific value of 1 kg of TDN and
* Calorific value of 1 kg of fat corrected milk

TABLE 31.1[a]

Milk Production Efficiencic' in Various Breeds of Cows and Buffaloes

Parameters	*Cross B X S*[b]	*Sahiwal*	*Buffalo*	*Desi*
Body Wt. (kg)	356	281	481	303
Milk yield (kg)/ 305 days	3300	2230	1740	400
Milk yield (kg)/day	9.68	7 46	5 79	1 32
Dry matter intake/per kg of Milk production	1.07	1 10	2 03	6.31
Dry matter intake (kg)/per day	10 38	8 20	12 12	7.77
TDN (kg) intake/per kg of milk production	0.65	0 66	1.35	3.51
Total Portein intake (kg) (305 day period lactation)	394	304	465	390
C. P intake (kg) per day	1.29	0 99	1 52	1.01
% of protein in milk	0.033	0 033	0.040	0 033
% Gross energetic efficiency of milk production	29 14	28.23	19.56	5.97
% Net energetic efficiency of milk production	51 20	51.13	37.57	12.22

a = Based on data from the National Dairy Research Institute, Karnal. (Annual report (1977) P 151)

b = B x S = Brown Swiss X Sahiwal crosses

Studies on the energetics of milk production in various Indian breeds of cows and other species like buffaloes and goats (*Table 3.1*) revealed that gross energetic efficiency of milk production in Brown Swiss X Sahiwal crossbred cows was of the order of 30 percent, buffaloes and Desi (Zebu) cows being 19.89 percent and 5.97 percent, respectively.

On the basis of the above results, it was also evident that 27 kg of dry matter will be required to produce 25 kg of milk from crossbred cows, while in the case of the Sahiwal, 28 kg of dry matter will be required to produce 25 kg of milk. On the countrary, buffaloes require 52 kg and Desi cows require 148 kg of dry matter to produce the same quantity of milk. Further, it has also been observed that less number of high yielding crossbred cows are required to produce the same, quantity of milk, that can be produced from low yielding *Desi* cows. Since overall cost of maintaining animals and labour involved in feeding and management will be less with a smaller number of high yielding crossbred cows, it will be always be a profitable proposition to possess high yielding crossbred cows rather than low yielding *Desi* cows.

Milk production efficiency in various breeds of goats (*Table* 31.2) also revealed that crossbred goats (Alpine x Beetal) gave maximum milk as compared to Alpine or Beetal goats. The efficiency of milk production in Alpine x Beetal crossbred goats was highest (25 percent) as compared to Alpine (21 percent) and

TABLE 31 2*

Milk Production Efficiencies in Various Breeds of Goats

Parameters	*Alpine*	*Beetal*	*Alpine X Beetal Cross*
A V. Body weight (kg)	53	49	52
Lactation length (days)	289	239	264
Lactation yield (kg)	437	271	488
Milk yield (kg)/day	1 51	1 13	1 85
D. M. intake (kg) days	2 06	1 90	2.14
TDN intake (kg)/day	1 16	1 12	1 25
Protein intake (kg)/day	0.35	0 31	0 37
D M intake kg/lit. of milk production	1.29	1 62	1.16
% gross energetic efficiency	21	17	25
Dry matter requirements producing 25 kgs of milk	34	42 0	29 0.

* Based on data from the National Dairy Research Institute, Karnal. (Annual Report 1977 P - 155)

Beetal 17 percent) goats. Thus, crossbred goats are economical in the sense that they require only 29 kg of dry matter to produce 25 kg of milk. The corresponding figures for Alpine and Beetal were 34 and 42 kg of dry matter, respectively.

The nature of the diet, for example, a mixture of 60 percent berseem hay and 40 percent straw, gave better energetic efficiency of milk production than only wheat straw and concentrate ration However, there are upper limits for this efficiency ratio. Not over one-half the consumed TDN energy can be converted into milk energy. Superior dairy animals convert about one-third of the consumed TDN energy into milk energy Good dairy animals convert about one-fourth (25 percent). Really profitable milk production involves higher dairy ment.

Apart from genetic factors, climate also plays a vital role in influencing milk production efficiency of cows. The efficiency of utilizable digestable energy at 21°C was 50 percent but went down to 40 percent when lactating cows were exposed to 32°C for a period of seven days. Marked decline in feed consumption and digestible energy was also noticed with a rise in environmental temperature. Apart from environmental temperature, another factor which influences milk production is body size The profitability of the dairy animal is, therefore, dependent upon its conversion efficiency which is influenced by the nature and type of feed genetic make up and size of the animal, and the climate to which the animal is exposed.

APPENDIX - I

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